THE MANUFACTURE OF COMPRESSED YEAST

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By

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PREFACE

THE rapid evolution of compressed yeast manufacture, from obscurity to a great industry, in the brief space of a few years writes another chapter in the romance of applied science. As a leavening agent, yeast was as well known in ancient Rome as it was to our civilization until, in 1861, the epochal researches of Louis Pasteur definitely established the *rôle* of yeast, its nature, methods of reproduction and the products of its action. Further research in fermentation phenomena proved that alcohol production is not essential to yeast growth, and this fact, coupled with the perfection of methods for the isolation of pure cultures, laid the foundation of the modern yeast industry.

During the first decade of the present century the application of science to the art was in its infancy; the widely used "barm" was a purely domestic preparation and the commercial yeast of the period was simply a waste product of the distillery and vinegar brewery. In the certain knowledge that theoretical yields were possible, hundreds of eminent workers toiled incessantly to apply Pasteur's discoveries in the development of non-alcoholic processes of yeast cultivation, and, as a result, yeast alone now forms the basis of vast and rapidly expanding industries in which possibilities exist that are unknown to many, even to those engaged in the allied fermentation and cereal industries.

In view of the importance of the subject it is strange that the investigator finds little mention of yeast production in our literature, and in contributing to a desideratum previously unsupplied, this book presents a comprehensive description of the various processes applied in yeast cultivation; a full explanation of the guiding principles of each process is given, with detailed examples to show how these principles are applied in practice at every phase of the process.

The raw materials and their treatment receive the attention

that their importance deserves, and the scope of the book has been extended to embrace a description of the plant, with specifications of the more important units, and an outline of the most suitable disposition of the vessels and fittings necessary for the economical production of compressed yeast.

F. G. W.

FEBRUARY, 1940.

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CHAPTER I

THE YEASTS, ETC.

The yeasts occur abundantly throughout nature and are often found as a creamy sediment in saccharine liquids in process of fermentation. If this sediment is examined under a high-powered microscope it is found to be composed of minute spherical, or slightly elongated, cells, either single or in pairs as mother and daughter cells, or a number may cling together, forming clusters. The individual cells may vary in diameter from 3 to 12 microns (1 micron $= \frac{1}{1000}$ mm.) according to variety, but the three essential cell constituents are always present—the cell membrane, protoplasm and nucleus.

The cell membrane is thick, clearly defined and acts as a protective covering, enclosing the cell contents under slight pressure. The protoplasm, a semi-fluid protein substance, is considered to be the centre of metabolism, digesting the food and producing the energy required to maintain the vital activities of the cell. The nucleus is small, but indispensable, and governs the physiological functions of the cell. The vacuole, often a prominent but relatively unimportant feature, consists of reserve material in the form of glycogen, which varies in size according to the nature of the food supply; or it may be non-existent.

There are innumerable varieties of yeast, differing widely in size and form, and in each variety the cells may differ slightly according to age, environment or concentration of nutriment when they were formed; but under standard cultural conditions each variety will show a normal range of dimensions. By their morphological and physiological differ-

ences they are divided into two main groups—the true and the pseudo-yeasts. The pseudo-yeasts resemble the true yeasts in many respects, but differ in that they reproduce by budding only. The true yeasts are budding fungi that, when conditions are favourable, will reproduce by the formation of endospores, and include many races with characteristics suited for their application to industry, and many others more or less harmful that injure the quality of fermented products.

The applied yeasts are distinguished by the products of their activities and divided into races known in industry as culture and wild yeasts. A race of yeast desirable in the distillery would, in that industry, be termed a culture yeast, but if it should develop in the ale brewery it would probably be regarded as a wild yeast. The use of the term " culture yeast " often implies a race adapted to the requirements of industry by a long process of selection and cultivation ; this assumption is not exactly correct owing to the fact that yeasts with identical characteristics may be isolated from the surface of ripe fruits and applied directly to the industry concerned. The races employed in manufactures have distinctive general properties which place them in one of the three following divisions :—

(1) Ale brewery yeasts produce moderate amounts of alcohol only, do not ferment dextrins, therefore they have little attenuating power, but rapidly form a compact sediment in the fermented liquid. Some types have a tendency to form a thick foamy layer of yeast at the top, others remain at the bottom of the liquid during fermentation, and are termed top and bottom yeasts respectively; there is no sharp line of distinction between the two types, however, and by suitable cultivation in appropriate media one form can be changed to function as the other.

(2) Distillery and compressed yeasts have great fermentative and reproductive power, ferment certain dextrins, produce large amounts of alcohol, have characteristics essential for rapid filter-pressing, and are valued for their resistance to climatic influences during transport.

(3) Wine yeasts comprise a very wide range of types, many of them differing in the aroma and flavour produced in the

fermented liquid. They are indifferent to high concentrations of alcohol and produce, in some cases, more than 18% from grape juice to which sugar is added.

Alcoholic Fermentation. The process of alcoholic fermentation marks the change in sugar, and decomposition products of sugar, which results in the formation of large amounts of alcohol. How the change actually occurs is not known because different fermentations occur simultaneously in the yeast cell and all the changes that take place are unknown, but it is considered that this, the almost quantitative conversion of sugar into alcohol and carbon dioxide, is probably the result of a series of reactions in which intermediate products are momentarily formed and used in succeeding stages of the process.

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6 = 4C_2H_5OH + 4CO_2$$

According to the above equation, 100 parts of cane sugar should produce 53·8 parts of alcohol and 51·46 parts of carbon dioxide. The actual yields from fermentation approach these figures very closely, but do not reach them because part of the sugar is assimilated by the yeast and the portion of the material thus removed from the reaction is not accounted for by the products of the fermentation. Under the most favourable conditions the total yield will rarely exceed 95% of the theoretical.

Pasteur showed that the fermentation of 100 parts of cane sugar (= 105·26 parts of dextrose) on the average produced 51·1 parts of alcohol and 49·3 parts of carbon dioxide. The alcoholic fermentation thus appears to be a rearrangement of the oxygen atoms within the molecule ; one part of the sugar molecule is reduced while the other part is oxidized. In the sugar molecule each carbon atom has one oxygen atom, but in the products of fermentation carbon dioxide has two oxygen atoms to one carbon atom, and in alcohol there is only one oxygen atom for two carbon atoms.

Attenuation. All the applied yeasts secrete and retain invertase and maltase in the cell, and, although they are capable of fermenting the sugars in the wort, the same wort fermented by different races of yeasts results in varying degrees

of attenuation. According to Fischer, the degree of completeness to which sugar is fermented depends largely upon the greater or less conformity of the geometrical structure of the sugar molecule and the enzymes of the yeast, and in a lesser degree upon the diffusibility of the sugar. The decomposition of the sugar takes place within the cell, and only so much sugar can ferment as reaches the protoplasm through the cell membrane.

The membranes of yeast cells differ in thickness according to type, and consequently do not permit the diffusion of sugar into the cell with equal facility, hence under equal conditions some yeasts will ferment the sugars more quickly than others and more completely in the same time. Yeasts have different powers of reproduction, different powers of resistance towards the products of fermentation, and are affected differently by aeration and temperature, all factors that exert an influence upon the completeness with which sugars are fermented by different yeasts under identical conditions. Attenuating power is therefore dependent upon the type of yeast and the physiological condition of the cells. It has been recently established that distillery yeasts of high attenuating power contain an enzyme having the property of inverting malto-dextrins to maltose.

Retardation. The products of fermentation check the activity of yeasts with an intensity that varies with the type of yeast and with the conditions. Different races show considerable differences in the degree of sensitiveness towards alcohol and the amount necessary to produce a given effect varies widely. The growth of certain types of ale brewery yeasts is definitely checked in an alcohol concentration of 3%, whilst a number of the wine yeasts are capable of active reproduction in the presence of 18% of alcohol in the substrate.

Under otherwise equal conditions a larger quantity of alcohol is necessary to check fermentative activity than to stop reproduction. Temperature exercises a determining influence on the amount required to retard fermentation, and this influence is progressive as the temperature rises, thus at a low temperature the final fermentation goes further, and the same amount of alcohol that then permits a slow fermentation

may kill all the cells as the temperature rises within the limits that can be taken into consideration.

Oxygen. The influence of oxygen in yeast metabolism was conclusively demonstrated by Pasteur in 1861, but this subject remained a matter of academic interest only, until the recent application of his discovery to the stimulation of yeast-cell multiplication in differential fermentations revived interest in it. Oxygen gas is not absorbed by yeast, only when it is dissolved in the nutrient medium; its solubility is very slight and amounts to about 0·0009% at 20° C. Free oxygen is not essential for the yeast to maintain its vital functions. When yeast is cultivated in a vessel filled with oxygen-free wort to which air has no access the yeast grows at the bottom of the liquid, and, finding a deficiency of oxygen, it uses a little of the sugar for maintaining itself and draws the energy it needs from the alcoholic fermentation ; under these conditions it will entirely suppress its reproductive functions, the rest of the sugar being transformed into alcohol and carbon dioxide by the enzymes, all the changes occurring without free oxygen taking any part in the reactions.

The activities of yeast are quite different when it is placed in wort supplied with sufficient oxygen for its needs ; in this condition it completely decomposes the sugar, using part for maintaining protoplasm and constructing new substance, transforming the rest by oxidation, as a source of energy, into carbon dioxide and water. In this case yeast is aerobic ; it is nourished, respires and multiplies like any other plant. No alcohol is formed and 190 parts of yeast may be produced from each 100 parts of disaccharide.

Yeast-Cell Reproduction. Under normal conditions reproduction proceeds by a process termed budding. The young bud takes the form of a simple protuberance, generally originating at the thick end of the cell and a little on one side of the apex of the oval outline which, in a greater or less degree, characterizes the cells of applied yeasts. Protoplasm enters the young bud, which speedily grows and assumes a spherical or oval form ; when it is quite large the nucleus divides by elongating in the mother cell until a portion enters the young bud ; this is followed by a median constriction,

and finally by a division at the constricted part, both portions then assuming the normal spherical shape in the mother and daughter cells. The bud continues to grow until the cell wall develops a tendency to meet at the point of attachment to the mother cell, the junction finally takes place and a partition forms between the two cells. The new cell is not always detached from the mother cell at once, but many cells may remain clinging together, forming clusters.

Under conditions of temperature and moisture-supply favourable to the process, the true yeasts form endospores. These are usually about half the diameter of the mother cell, and from one to eight may occur in each cell. Abundant moisture, very little food and low temperatures favour spore formation. Hansen has used the difference in the time and temperature limits of spore formation to differentiate between the various types of true yeasts.

Nutrition. All living cells require food for the maintenance of life. Yeast can only assimilate substances that are in solution and able to diffuse through the cell wall. It builds up its protoplasm from proteins in the form of amino acids or peptones, usually derived from the "native" proteins by hydrolysis. Yeast is also included in the group of autotrophic micro-organisms that assimilate inorganic nitrogen and grow with nitrates, ammonia or ammonium salts as the only source of nitrogen, producing from the simplest materials the complex organic substances found in the cell, decomposing sugar at the same time to provide the energy required in the process.

Yeast can be caused to ferment without reproduction when placed in an oxygen-free solution of nutrient medium contained in a closed vessel, and, on the other hand, it can be compelled to grow without exerting any fermentative activity if grown in a nutritive solution in the presence of a sugar it cannot ferment, such as lactose. On the commercial scale the same result is obtained by the intense aeration of extremely dilute worts containing inorganic nitrogen.

In many cases the microscopic appearance of yeast corresponds to a specific physiological state, and the conditions under which compressed yeast has been cultivated may often be deduced with a certain amount of accuracy.

Yeast from feebly aerated spirit worts of high initial gravity (12° Balling) fermented at low temperature are large and spherical with a highly refractive plasma, in which the vacuole is large but often ill-defined. The protein is invariably high, between 54% and 58% of the dry substance.

Yeasts from the more strongly aerated spirit worts of low initial gravity (1°–5° Balling) are normal in diameter with a clearly defined vacuole, and if fermented at high temperature small granules will be found embedded in the protoplasm. The protein content varies with the nutrition and intensity of aeration.

Yeasts from the turbulently aerated differential fermentations average about two-thirds the diameter of the normal cell, are less uniform in size and appear to be slightly flattened on one side. The protoplasm is translucent and the vacuole small and sharply defined. The protein varies between 50% and 52% of the dry substance.

Temperature. The activity of yeast is entirely dependent upon the temperature, and as the cells have the same temperature as their environment, the velocity of reproduction and fermentation is readily controlled by temperature variation. Yeasts survive exposure to very low temperatures; they may be preserved without injury by freezing and show slight signs of reproductive as well as fermentative energy at 4° C. Yeast activity increases with increasing temperatures, exhibiting the greatest energy at 30° C. Beyond this point the rate of growth decreases rapidly and ceases entirely at 42° C. Above the optimum temperature the cells produced are abnormal, degenerate rapidly, and tend to involution forms with strongly granulated plasma.

Carbon Dioxide. The amount of CO_2, gaseous or dissolved, in the wort during fermentation depends mainly upon the intensity of aeration. In a turbulently aerated differential fermentation the CO_2 is dissipated as rapidly as it is formed and its physiological influence upon the yeast is negligible, but if fermentation is conducted in a closed vessel saturated with CO_2 under pressure the growth of yeast and fermentative activity are both retarded at the same time.

Water. Yeast contains 73% water and 27% solid matter.

Its food is obtained by diffusion through the cell membrane, and therefore it can only be assimilated when it is in solution. The minimum amount of water in which yeast may be successfully cultivated is difficult to estimate, and depends largely upon osmotic pressure and the solubility of the nutriments.

Yeast Ash. With the exception of the determination of carbon, nitrogen, and moisture, knowledge of the chemical composition of yeast is of little service from the commercial standpoint in estimating the relative value of bread yeasts; this feature depends more upon physiological properties such as fermentative and attenuating power and durability, for which appropriate tests are employed; but chemical analyses of both yeast and substrate are essential when yeast is to be differentially nourished in worts deficient in essential inorganic materials. The deficiency is then corrected by the addition of a calculated amount of the substances required; for instance, cleared molasses require the addition of phosphates, and worts produced from tapioca roots or sugar may lack potassium salts and phosphates.

When propagated in worts deficient in mineral matter the yeast adapts itself, within certain definite limits, to the nature of the nutriment and produces cells relatively poor in ash until one of the essential inorganic constituents becomes exhausted and yeast growth then ceases. It is due to this feature that the ash of yeast may vary so widely in amount and composition, but it is only in extreme cases that the physiological characteristics are materially affected.

The moisture content of compressed yeast in a saleable condition is remarkably constant at 73% and a variation of 0·5% either way is immediately apparent in the consistency of the yeast. Yeasts produced from scientifically controlled differential fermentations show very little variation in either protein (average 51·25%) or carbon (average 42·5%), but the ash differs widely according to the inorganic constituents of the wort. The addition of molasses to wort derived from grain is immediately followed by an increase in the ash content of the yeast propagated in the mixture. For this reason cereal worts are adopted as the standard substrate in which yeast is grown for yeast-ash analysis.

The items shown below represent the mean of thirty different analyses of compressed yeast in which the mineral matter averaged 7·36% of the dry substance, and the chief constituents, phosphoric and potassium compounds, varied but slightly in the different samples.

K_2O = 31·45%	SO_3 = 5·47%
P_2O_5 = 50·10%	SiO_2 = 1·40%
MgO = 3·05%	Fe_2O_3 = 0·38%
CaO = 5·69%	Na_2O = 1·39%

Varieties of Yeast. A detailed description of the numerous known races embraced in each variety of yeast is of little value as a means of identification owing to the remarkable variation in the general properties of any one race that follows a slight modification in either nutrition or environment.

In describing the more prominent features that distinguish the different varieties a generalization only is possible, because there is no sharp line of distinction and the properties of one variety will often merge with those of another. In the identification of yeasts, microscopic observation may be sufficient in the case of yeast with some distinctive feature such as the hat-shaped spores of *Sacch. Anomalus;* otherwise microscopic examination must be supplemented by the isolation of pure cultures and examination of the yeast and substrate after cultivation under standard conditions.

Saccharomyces Cerevisiæ. This is the most important variety of all yeasts, and includes the innumerable races used in the cereal fermentation industries as bread, beer, and distillery yeasts. In general they are characterized by the efficient conversion of sugar to alcohol, rapid settling after fermentation and the production of fermented liquids clean in flavour and in appearance. The species is universally used in bread-making and the domestic arts, and forms the base of many great industries.

It is described at length in this volume where, unless otherwise specified, the term " yeast " is used to indicate a selected race of the variety used in the manufacture of compressed yeast.

Saccharomyces Ellipsoideus. This is the true wine yeast,

occurring chiefly on ripe grapes ; and a number of the many races embraced in this variety may be found on the same grapes. Several species are noted for their great fermentative power and are of importance in the fermentation of must and of fruit juices for wine, cider and vinegar manufacture and in alcohol distilleries.

The cells vary in shape from spherical to oval, and it is only occasionally that they are elongated. Spores are readily formed on gypsum blocks within twenty-two hours at 30° C. Most races of the variety produce large amounts of alcohol and some are capable of active reproduction in the presence of 18% alcohol. A characteristic vinous, or wine-like flavour is produced in the fermented liquids, and when the sugar is decomposed the cells settle rapidly to form a compact white sediment.

Sacch. Ellipsoideus ferments saccharose, maltose and dextrose. Pure cultures are often employed in distilleries, but the variety is undesirable in ale breweries where it produces a persistent turbidity and disagreeable bitterness in beer.

Saccharomyces Apiculatus. This variety is widely distributed in nature and particularly abundant on ripe grapes. It is often found active in the first stages of the spontaneous fermentation of fruit juices and of must, producing from 3% up to 5% alcohol, which appears to be the limit of its alcoholic tolerance ; in some cases the fermentation is then continued by *Sacch. Ellipsoideus* with the destruction of *Sacch. Apiculatus* by the higher concentration of alcohol.

The classification of this species as a true yeast was doubtful until recently as the spores are formed with considerable difficulty. The cells are smaller than most true yeasts and vary in shape from oval to elongated forms with an apiculation at one or both ends. The apiculations are the buds of daughter cells and the rounded cells are the cells after division.

Sacch. Apiculatus causes a persistent cloudiness in beer and spoils the flavour of wine ; it is a sedimentary yeast and ferments dextrose but does not secrete invertase ; consequently it cannot ferment saccharose or maltose.

Saccharomyces Pasteurianus. Many varieties of this yeast are found on fruit and grapes, consequently they take an

active part in the fermentation of fruit juices and of must when the temperature is favourable. Its presence is undesirable, however, and results in the production of inferior wine. Several forms are found as an injurious yeast in breweries, where it causes turbidity and a peculiar bitterness in beer and in beverages prepared from fruit juices. The cells of the species vary in shape, according to environment and substrate, from oval to cylindrical, sometimes producing a branching mycelium in film formation. It forms spores in twenty-five hours at 25° C. and ferments saccharose, maltose and dextrose.

Saccharomyces Ludwigii. This variety may be found on ripe fruits and fermenting fruit juices. It ferments dextrose, producing up to 10% alcohol from this sugar, but it does not secrete maltase, and under the most favourable conditions produces only 1·2% alcohol in malt worts. The cells are exceptionally variable in shape and size, and when lemon-shaped bear a resemblance to *S. Apiculatus*, but differ in that they are much larger.

Saccharomyces Maleii. This yeast is often found on the surface of ripe apples and is isolated in pure cultures for use in the manufacture of sparkling cider. The cells resemble *S. Ellipsoideus*, but are much larger and produce only moderate amounts of alcohol at a slower rate of fermentation.

Saccharomyces Saké. Is extensively used in the Orient for the fermentation of worts derived from rice in producing saké and whisky. It is noted for its energetic fermentation and the production of high concentrations of alcohol, in some cases exceeding 20%. The cells vary in shape from round to elongated forms, spores are produced abundantly in fourteen hours at 30° C.

Saccharomyces Anomalus. Develops as a heavy white film on the surface of saccharine liquids. It is easily recognized by the formation of spores with a peculiar zonal ring resembling a hat. Spores are formed in nineteen hours at 30° C. The cells are small, oval to elongated in shape and in microscopic appearance resembles *torula*. It is often troublesome in distilleries and forms small amounts of alcohol. *S. Anomalus* ferments dextrose but cannot ferment maltose or lactose, and secretes scarcely any invertase. It is noteworthy because it

produces relatively large amounts of esters and decomposes alcohol to form CO_2 and water.

Pseudo-Yeasts. *Mycoderma.* The innumerable varieties of yeasts embraced in this group are divided into two sub-groups—*Mycoderma Vini*, found on wine and fruit juices, and *Mycoderma Cerevisiæ*, found on alcoholic liquids derived from cereals.

If wine or wort of low alcohol content is freely exposed to the air at 27° C. it will be covered, in about three days, with a smooth chalky film. In a few weeks this becomes thick and folded, and at the same time develops a more or less fruity ester odour. This film is composed of the oval or elongated cylindrical cells of the strongly aerobic mycoderma yeasts that develop only on the surface of liquids in full contact with air, forming the large aggregations known in industry as wine or vinegar " flowers."

The cells are reproduced by budding only and are characterized by the vigorous and complete combustion of alcohol, organic acids, and sugars to CO_2 and water. Pure cultures in alcoholic wort destroy all the alcohol and eventually change the substrate from an acid to an alkaline reaction. In the earlier primitive methods of air yeast manufacture the mycoderma yeasts were often troublesome, particularly when worts of high *p*H were fermented for the production of seed yeast, and an abundant air supply provided these aerobes with the optimum conditions for rapid development.

Torula. This group embraces many species of yeast with widely different properties, having in common only the characteristics of sedimentary growth and lack of spore formation. The cells vary in size from 1·5 to 5 microns, and in shape from spherical to more or less elongated forms, reproducing by budding and in rare cases by mycelium formation. Torula cannot invert saccharose and a few types only secrete maltase, producing a feeble alcoholic fermentation. Several varieties are noted for the production of red and green pigments.

Moulds

Moulds. These are distinguished by the formation of a cotton-like structure called the mycelium. This consists of a

network of filaments, or threads, each of which is called a mycelial thread, or hypha, and may be either vegetative or fertile. The vegetative hyphæ secure and assimilate the nutriment and eliminate excess moisture.

The fertile hyphæ, or conidiophores, are those which bear the fruiting body and generally produce enormous numbers of spores, each of which may develop into a typical mould plant. In a few types the spores may be sexual, but most are asexual and formed at the end of the conidiophores.

The structure of the hyphæ differs in certain species of the moulds, some are composed of many cells divided by cross walls, or septa ; in others these septa have not been observed and their presence or absence is a factor in identifying the species. Moulds differ from each other principally in their methods of producing spores and conidiophores, and in many types there are easily recognizable differences in the appearance of the mycelium.

Certain species of moulds are capable of growing within very wide limits of temperature and variety of nutriment, but as a rule they require plenty of air and moisture. They are constantly found upon every kind of putrescible matter and are particularly active in the fermentation and decomposition of foodstuffs.

A few species have acquired importance in industry due to their diastatic and fermentative activities, other types are utilized in the manufacture of citric acid and in the ripening of cheese. Many varieties find conditions favourable for their growth in the materials and vessels used in the cereal industries, and if not suppressed they multiply at an alarming rate and interfere with the processes and the quality of the product.

Penicillium. Most of the green moulds are members of the universally distributed *Penicillium* group. They thrive on a diversity of materials ranging from fruit juices and carbohydrates to moist leather. They are autotrophic and grow well in saccharine solutions with inorganic salts as the only source of nitrogenous nutriment.

The mycelium is white in young colonies and composed of loosely woven hyphæ, changing in colour to blue or grey-green with the formation of spores ; a dark green or brown tint

distinguishes old colonies. The spores are spherical and formed in abundance upon conidiophores arising either from submerged hyphæ or as branches of aerial hyphæ. The fructifications consist of a complex system of branches, usually grouped near the upper end of the conidiophore giving the appearance of one or more brooms or brushes, the cells producing chains of spores by constriction.

Penicillium Glaucum, or *Expansum*, is the best known member of this group. It is commonly found in the fermentation industries and is particularly objectionable owing to its disagreeable mouldy odour. Starches and saccharine substances with a slight acid reaction appear to be the most favourable medium for its growth. It is most active at temperatures ranging from 15° to 25° C., and is destroyed at 45° C. Other well-known varieties of this group include *Penicillium Roquefortii* found in pure culture in Roquefort cheese, and *Penicillium Camembertii* the active agent in ripening Camembert cheese.

Aspergillus. This group includes numerous varieties found in every country, and although they develop under widely different conditions and thrive on a wide range of materials they are easily recognized by their method of spore formation. The upright conidiophores are unseptate, generally much larger than the vegetative hyphæ, and terminate in an abrupt enlargement bearing numerous chains of spores over the whole of its surface.

Aspergillus Niger. In young colonies the growth is at first white and filamentous, but spores are formed in such abundance that the growth soon becomes black in colour. The species is noted for the fermentation of sugar solutions with the production of oxalic acid, crystals of which are visible in the mycelium by microscopic examination.

Aspergillus Glaucus. This species develops rapidly on suitable media over a wide range of temperatures and shares with *Aspergillus Repens* the property of forming bright yellow fruiting heads, called perithecia, in such abundance that a yellow colour is imparted to the culture. *Aspergillus Glaucus* causes considerable damage to grains during the malting process.

Aspergillus Oryzæ is applied in the production of Taka diastase and is used in pure culture for starch hydrolysis in the Saké industry of Japan. Spores are cultivated on soaked rice, and after drying, are used to inoculate gelatinized rice. The mould converts the rice starch to sugar, and this is fermented for the production of saké. Amylase is produced by other members of this group, but in a lesser degree.

Mucors. The black moulds are associated with the decay of many kinds of food stored in humid situations: stale bread, if moist, invariably develops a vigorous growth of Mucor, and on this account the mucors are often termed bread moulds. Owing to their strong diastatic power several of the mucors are of great industrial importance.

In saccharine liquids under aerobic conditions yeast-like cells are produced which convert sugar into alcohol and CO_2. The fermentative power varies greatly with the species, but the efficiency of some approaches the more active yeasts, very few moulds possess this characteristic.

In the moulds of this group numerous spores are borne in spherical sacs at the end of erect fertile hyphæ; septa develop in the mycelium only when sporangia begin to appear.

Mucor Rouxii is the most active of the species in transforming starch to sugar and is extensively employed in Europe in what is known as the Amylo process. On solid media it develops as a mould and in liquids as a yeast. Several races are applied in the manufacture of alcohol from cereals. A number of well-known varieties of the Mucors are employed in the fermentation industries of Asia, including *Mucor Mucedo*, *M. Circinelloides*, *M. Racemosus*, *M. Javanicus*, *Rhizopus Oryzæ* and *Rhizopus Javanicus*.

Dematium. This variety is not active as an agent of fermentation, but members of this group are often found in the fermentation industries as a whitish-grey slimy colony changing later to a leathery growth of black colour. One species, *Dematium Pullulans*, is frequently found as a dark brown colony in decaying fruits. In wort it develops as yeast-like cells that reproduce by budding. In cultures an irregularly shaped greyish-green mycelium is sparingly produced, and spores are borne in clusters all along the hyphæ submerged in the substrate.

Oidium. The members of this group are universally found on milk, fruits, cereals, and on the materials and utensils used in the brewery; they produce only a small amount of alcohol in malt worts. No specific fruiting body occurs on this mould; spores are formed by segmentation and division of the aerial hyphæ to form chains of barrel-shaped cells termed "oidia." Under conditions favourable to the process the whole mycelium will divide into abruptly cylindrical oidia, the appearance of which serves to identify the species. Colonies of *Oidium Lactis* are colourless in pure cultures. In liquid media the vegetative mycelium is entirely submerged and often produces a strong characteristic odour. On solid media it forms a dry white film. The exposed surfaces of compressed yeast in bulk may become powdery-white and dry with the matured spores of oidium.

Monilia. This group represents a stage in the growth of sclerotinia, but some of the members of the Monilia group do not produce all the forms comprising the complete cycle of sclerotinia, and are therefore classed as imperfect fungi. They are parasitic on fruits, and, amongst other undesirable characteristics, cause the disease known as brown rot. *Monilia Candida*, a well-known member of the group, appears as a yeast in young cultures in malt worts, but later develops a mycelium. On solid media, oval or elliptical cells are produced by segmentation of the hyphæ. This form readily ferments saccharose and maltose over a wide range of temperatures up to 40° C. and produces 5% alcohol in malt worts.

Bacteria

Discussion of fermentation bacteria of interest to the fermentation industries under review may be confined to two applied groups—lactic acid bacteria and acetic acid bacteria.

Lactic Acid Bacteria. In addition to the recognized lactic acid producing micro-organisms, there is a great number, and amongst them some pathogenic varieties, which develop this acid by the fermentative decomposition of various sugars, including saccharose, maltose, lactose, mannite and sorbite. Many bacteria produce lactic acid as a subsidiary product, but when sugar is decomposed as a source of energy by the

bacteria found in industry this acid is the chief product. This group of organisms differ widely in form and physiological properties; some occurring in milk are motile, while those found in cereal products vary from pediococcus types to short rods and are not motile. They change sugar to lactic acid only; no volatile acids or gases are formed, and spores are produced by a few of the species only.

The different races, which include *Bacillus Acidi Lactici*, *Bacillus Lactis Aerogenes*, *Streptococcus Acidi Lactici* and *Micrococcus Acidi Lactici*, vary greatly in their properties; some resist a temperature of 60° C. and still remain active, others are sensitive to temperature variations and cease both reproduction and acid formation above 37° C.

The type of importance in the compressed yeast industry is most active at about 50° C., a temperature at which most of the micro-organisms occurring in the cereal industries cease to function, consequently by maintaining a mash temperature at 50° C. a nearly pure lactic acid fermentation is obtained by temperature selection.

In malt worts lactic acid is produced by the inversion of maltose to dextrose and the decomposition of this sugar to lactic acid according to the equation,

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6 = 4C_3H_6O_3.$$

The lactic acid thus formed possesses considerable latent heat, and at temperatures ranging from 35° to 40° C. may be further decomposed to butyric acid by the anaerobic butyric organism. Concentrated lactic acid is a colourless syrupy liquid with an intensely sour taste; it is not volatile and is the characteristic acid of milk and beer.

Acetic Acid Bacteria. Acetic acid is produced in small amounts by quite a large number of micro-organisms, but the common vinegar bacteria are characterised by their ability rapidly to convert large amounts of ethyl alcohol into acetic acid and in concentrations that may approach 10% acid; but these bacteria are able to use their own product as a further source of energy, and if the process is allowed to proceed the oxidation is continued until the acetic acid is decomposed to CO_2 and water.

Several distinct forms of bacteria are applied in vinegar manufacture, and they may be found distributed throughout the liquid in such numbers that the gyle has a silky streamlined appearance.

On liquids of low alcohol concentration standing at room temperature, the bacteria cohere in gelatinous masses to form the slimy, tough and almost transparent film known as " mother of vinegar." If the liquid is undisturbed the film remains on the surface until all the alcohol is changed to acetic and water. A very slight disturbance causes the film to sink, a new film forms, and with occasional disturbance a succession of films form a submerged gelatinous mass in the vinegar.

Acetic acid bacteria do not attack sugars to any appreciable extent, and in vinegar production the alcoholic fermentation by yeast must precede that of the acetifying bacteria. The two fermentations cannot proceed simultaneously for the reason that the acetic acid destroys yeast with the result that unfermented sugar remains in the vinegar.

The optimum temperature for acetic acid bacteria ranges from 30° to 40° C. No acid is formed below 15° C. and reproduction ceases at 10° C. They are strictly aerobic and active only when freely supplied with oxygen. Four well-known varieties are found in the vinegar industry.

Bacterium Aceti consists of short rods about 2 microns in length, slightly constricted in the middle or irregularly swollen, they often occur in long chains, not motile, do not form spores, and are stained yellow with iodine. The cells of *Bacterium Pasteurianum* are larger than those of *Bact. Aceti*, but are produced in thread-like and involution forms. They are not motile and the gelatinous sheath is stained blue with iodine. The cells of *Bact. Kutzingianum* and *Bact. Xylinum* resemble those of *Bact. Aceti*, but more often occur individually or in pairs. They are both stained blue with iodine.

Other varieties, such as *B. Acetigenous*, *B. Oxidans* and *B. Industrious*, occur in alcoholic wash and differ from the above types in that they are motile and form acids from a large number of sugars and related substances. They are facultative aerobes and may be found in alcoholic wash stored for sedimentation as straight rods, either singly or in pairs.

They decompose carbohydrates with such energy that the total acidity of poorly attenuated wash may be increased by as much as 1% in a few weeks.

Enzymes

A number of soluble substances found in plants and animals possess the remarkable property of changing complex organic compounds, in the presence of moisture, to simpler substances without undergoing any change themselves. These converting agents, known as " enzymes," are divided into groups according to the compounds they act upon and the nature of their action. Those acting upon starch are called amylolytic, on proteins proteolytic, on fats lypolytic.

Enzymes are remarkably specific in their activities and act only upon a substance, or group of substances, having similarity in chemical composition. It is possible that the enzyme first unites with the substrate, and to do this it must have a configuration corresponding with that of the substrate. In many cases the conversion commences with the assimilation of water hence it is obvious that the enzymes of this group are primarily hydrators, and in consequence they are divided into two sub-groups—the hydrolytic enzymes of carbohydrates, embracing cellulase, diastase, invertase, lactase and maltase, and the hydrolytic enzymes of proteins, protease, peptase and tryptase.

The zymases, alcoholase and lactacidase, have the property of decomposing sugars as a source of energy. Other groups of enzymes are active in oxidation, in reduction and in coagulation.

As indispensable factors in biological processes the enzymes are found in all plant and animal tissues. Those contained in the animal system change the complex nitrogenous compounds to peptones, polypeptides and amino acids and the carbohydrates to simple sugars before assimilation. Some of the moulds secrete corresponding enzymes and thrive on starch and insoluble albumen.

Living cells produce a number of enzymes that are distinguished by their synthetic or analytic properties. In some cases their action depends upon other substances called

co-enzymes. In the alcoholic fermentation alcoholase can only act in co-operation with phosphates and an unknown substance that is diffusible and not destroyed by boiling. The activities of the hydrolytic enzymes are apparently directed to the preparation of food by changing insoluble substances into diffusible modifications which can then pass into the cell where digestion, and the final changes which liberate energy, take place.

In the yeasts enzymes are retained for the conversion and assimilation of food within the cell. Many of these changes are considered to be of a chemical nature ; for several of them the chemical equations are well known, and the conversions may be produced by chemical means without enzymes taking any part in the process.

Their chemical composition is uncertain owing to the difficulty of preparing them in a state of purity suitable for examination ; they lose in activity the more they are purified or separated from the albuminous bodies which accompany them. Enzymes acting upon certain of the carbohydrates are possibly of a carbohydrate nature, and the proteolytic enzymes are either proteins or compounds so readily absorbed by proteins that it is impossible to separate them.

Enzymes are soluble in water, colloidal and non-diffusible. They are most active at certain optimum temperatures, which generally range between 45° and 55° C. Their activity is retarded by cooling and is completely destroyed at 85° C. They act independently of the cell which produces them, but they may be inactivated by an accumulation of the products of their activities.

Enzyme activity depends upon a strictly limited hydrogen ion concentration in the substrate. Thus, in addition to an optimum temperature, there is also an optimum *p*H value for each enzyme ; for instance, the diastatic conversion of starch is most efficient at *p*H 5 and invertase is most active at *p*H 4·5.

The separation of enzymes from the aqueous plant or animal extracts in which they are dissolved is difficult and requires considerable experience. The crystalloids are removed from the solution by dialysis, the enzyme is precipitated with

alcohol, filtered from the solution and washed with alcohol, and then dried to a soft white powder *in vacuo*. The separation generally results in a considerable loss of activity which is increased by any further purification process.

Diastase, or Amylase, is one of the most common enzymes in nature. It is found in all green plants and in germinating seeds; it decomposes the starch to feed the embryo plant. Many of the moulds secrete this enzyme, but with few exceptions the yeasts and bacteria cannot attack starch for lack of diastase.

When placed in contact with starch at temperatures ranging between 40° and 70° C. diastase rapidly changes the starch into dextrin and maltose. The process is of an exceedingly complex nature, dextrin and maltose being produced simultaneously from the starch without any diminution, in amount or activity, of the diastase. The starch molecule is disintegrated in successive stages, at first a molecule of maltose is formed and a molecule of dextrin having a molecular weight nearly as high as starch. This dextrin is in turn split into maltose and a dextrin of lower molecular weight, and the process continues until a dextrin residue is left on which diastase does not act under usual conditions.

It is this change of the starch into products soluble in water that constitutes the conversion of starch in the mashing process, but the temperatures employed have a determinating influence upon the relative amounts of maltose and dextrin produced. The proportion of maltose is invariably higher when conversion takes place below 62° C.

Cytase, or Cellulase, decomposes cellulose into soluble sugars. It is active in dissolving the cellulose envelope surrounding the starch granule, and in germinating seeds liberates the starch granulose, which is then decomposed by diastase to furnish nutritive material for the young plant.

Cytase may be extracted from green malt with cold water, but it is an enzyme quite difficult to obtain and far more sensitive to heat than diastase; its activity diminishes at 60° C. and is destroyed at 65° C.

Invertase, or Sucrase, yeast cannot directly ferment saccharose, but secretes an enzyme called invertase which splits cane sugar into the two fermentable monosaccharides,

dextrose and lævulose, known as invert sugar. The inversion is influenced, more or less, by the concentration, the velocity increasing with the strength of the solution up to 20%. Invertase is a very common enzyme found in all green plants and in many yeasts, moulds and bacteria. It acts upon saccharose only and may be obtained as a white powder by extracting yeast with water, precipitating from this solution with alcohol and drying over sulphuric acid. It is easily soluble in water, acts only in acid solution and does not lose in activity by drying.

Maltase is secreted by many yeasts and moulds, and changes the disaccharide maltose into dextrose by hydrolysis. Like other endo-enzymes, it can only be extracted from the crushed and ground air-dried cells by leaching with water, but it is inactivated by precipitation with alcohol. Maltase exhibits its greatest activity at 40° C. and is destroyed at temperatures between 50° and 55° C.

Melibiase. This enzyme decomposes melibiose into dextrose and galactose. It is soluble in water and may be extracted from certain varieties of yeast by leaching the dried cells with water.

Lactase splits lactose into galactose and dextrose. It occurs in a few yeasts only, but never in brewery or distillery yeasts. It does not diffuse or penetrate the cell wall.

Zymases are the enzymes, such as alcoholase, lactacidase and vinegar oxidase, that provide the energy for cell life by causing fermentative decompositions.

Alcoholase, the zymase of yeast, splits sugar into alcohol and carbon dioxide. Buchner discovered this enzyme in yeast juice, obtained by grinding yeast with sharp sand to rupture the cells and subjecting the mass to great pressure. After removing the solids in a centrifuge the clear solution is found to have the same power as yeast in decomposing sugar to alcohol and CO_2. The solution is very sensitive, and when heated to between 35° and 38° C. the enzyme loses its power and begins to coagulate. It may be evaporated to dryness at low temperature and preserved without loss of activity.

The percentage of alcoholase in yeast varies with the cultural conditions. It is lowest during fermentation and increases sharply during the maturing period at the end of fermentation.

Oxidase. Enzymes of this class are considered to be active in the oxidative changes induced in a number of organic substances, such as sugars, aldehydes, alcohols and fats. The oxidase secreted by acetic acid bacteria is well known for its energetic oxidation of alcohol to acetic acid in the manufacture of vinegar. Yeast oxidase is assumed to take an active part in building plasma and autofermentation. Its presence is indicated by the heat generated when air is passed through finely divided yeast.

Endotryptase. The proteolytic endo-enzyme of yeast is able to hydrolyse the protein molecule to amino acids and basic substances. It is considered to be active in the digestion of protein food, the formation of plasma and in regulating the protein content of the cell.

Endotryptase is precipitated from yeast juice with alcohol, but cannot be separated from invertase. It is most active at 45° C. in 0·2% solution of hydrochloric acid and is destroyed at 50° C. Endotryptase plays an important part in the autofermentation of yeast.

Peptase. This enzyme acts upon the proteins of malted grain, changing them by hydrolysis into peptones and polypeptides. Its action is promoted, in the mash, by the presence of a small amount of lactic acid. Peptase may be extracted from green malt and is most active at temperatures ranging from 38° to 54° C. It is destroyed at 68° C.

Brewing Materials

Starch in the form of tubers and cereals, and proteins supplied by malt culms and cereals, are the two essential raw materials, plus water, required in brewing for the production of yeast; but in many cases these substances are supplemented by the addition of molasses or other saccharine materials. Some of the cereals are used in the form of malt, as wheat or barley malt, but the greater proportion of the material used in mashing consists of unmalted grain, usually referred to as raw grain, or tubers in the form of potatoes or dried tapioca roots.

From these materials the wort receives the three essential constituents for yeast production. Sugar from the decomposi-

tion of starch. Diffusable proteins from the insoluble native proteins, and small amounts of mineral matter. These substances extracted from the grain by the water with the aid of enzymes are included in the all-embracing term "extract."

Starch, or Amylum, occurs to a greater or less extent in all plant life except the lowest, such as algæ and lichens. It is distributed through all parts of the vegetable structure except the epidermis, but is found most abundantly in the seeds of cereals at the end of the vegetation period, and in certain parts of other plants such as the medulla of the sago palm, the rhyzome of the maranta and canna and the tubers of the potato and tapioca where it accumulates as a store of nutriment to be drawn upon by the embryo.

The chemical composition of starch is always the same irrespective of its origin, and consists of oxygen, carbon and hydrogen. It is one of the primary accumulation products of green plants, in the cells of which it is formed from water and carbon dioxide by the action of light. It is assumed that formaldehyde, the first product formed, is transformed into sugar which migrates to the seeds or tubers of plants and is there deposited in the form of transitory starch.

Starch is a white glistening powder, it is tasteless and insoluble in cold water, ether or alcohol, but if a mixture of starch and water is heated to a temperature, which differs with the origin of the starch, the granules burst to form a paste or opalescent solution. The higher the temperature the more quickly will the starch gelatinize. In boiling water the starch will form a paste more quickly than in water at 75° C., and in water at 120° C., when heated under pressure, it gelatinizes more quickly than at the temperature of boiling water. The more finely divided the starch the quicker it will gelatinize and the more flinty its nature the slower it will gelatinize.

The time required for the complete conversion of starch to maltose depends, in the mash tun, upon the rapidity of gelatinization as the diastase acts only upon gelatinized starch, so that although at 55° C. the diastatic activity is very great, complete conversion is not so quickly attained as at 62° C. where the energy of the diastase is diminished but the rapidity of gelatinization is very much increased.

Starch granules have a concentric stratified structure, layers of different constitution being superimposed one upon the other; the granules consist chiefly of a substance termed granulose, together with a closely allied substance termed starch cellulose. The shape and size of the starch granules are often highly characteristic of the plant by which they were produced, and this feature often serves to identify the presence of starch from some particular source.

To one familiar with the morphological differences of the various starches microscopical investigation affords the simplest method of ascertaining the origin of any particular starch. As the minute points of difference are almost incapable of description or delineation, the safest method, in any case, is to prepare a collection of standard specimens of the different varieties of starch for comparison with the samples to be tested, making the observation under identical conditions as regards magnification and illumination. As the largest of the starch granules are very minute, the microscope should be capable of a magnification of at least 500 diameters.

Proteins. Although proteins form the principle constituent of the animal organism they are produced exclusively by the plants and are found chiefly in the seeds. When absorbed in animal tissues they are only slightly modified so that the animal and vegetable proteins have the same general properties, yielding on hydrolysis a mixture of the same amino acids.

In some respects the degradation of the proteins is analogous to that of the polysaccharides except that the digestion process is far more complex than in the case of the polysaccharides which at most yield three monosaccharides, whereas the proteins give rise to a large number of amino acids after complete hydrolysis.

Plant proteins are classified, according to their properties, as albumens, globulins, glutelins and prolamines. Seeds naturally contain both the reserve proteins of the endosperm, and in smaller quantities the protein of the embryo, consequently a mixture of proteins is extracted from the crushed grain during the mashing process, and these are attacked by the enzymes to produce hydrolysed proteins with characteristic properties.

Cereals. Wheat, barley, oats, rye and rice are botanically included in the order of grasses and, like most of the wild grasses, the cereals are hermaphrodite, each blossom bearing both male and female elements, though often one or the other is abortive. Wheat and barley have no wild prototypes and their origin cannot be traced beyond their cultivated forms. The same is true of the other cereals all of which have been under cultivation from prehistoric times. In the grasses the germ is always provided with a store of nutritive material to ensure its support during the period of germination, and it is in this accumulation of reserve substances that mankind finds an abundant supply of food.

A section of a grain made lengthwise through the furrow which runs the length of the grain, will show it to consist of the following parts. The outside is a thin yellowish, or brown, skin surrounding the starchy material which forms the bulk of the grain. If a section of this is examined under a low-powered microscope, this skin is seen to consist of several layers differing slightly in structure. The first three coats constitute the epidermis, or cuticle, the epicarp and endocarp forming the next two layers are composed of finely reticulated cells. Immediately beneath the endocarp is the true skin, termed the episperm, or testa. The next series of cells constitute the aleurone layer, rich in protein and fatty substances.

The germ, located at the lower end of the grain, is a rudimentary plant preformed in all its parts, including leaves, stem and roots, and contains most of the fat present in the grain. The three parts of the germ are known as the plumule, consisting of the undeveloped leaves of the future plant ; the radicle or root, being quite distinct, and the third part the scutellum, which performs the important function during germination of conveying the nutritive substances from the endosperm to the young plant.

Wheat. *Triticum Vulgare.* This is a true grass, a cultivated annual unknown in the wild state, and there is no grass which can be regarded as its parent form. In germinating from the seed it first appears as a single blade and is therefore a monocotyledon. Its later development is endogenous.

Wheat may be cultivated in all latitudes, from the tropics

to the limits of the temperate zone, but it is best suited to soils of an argillaceous nature. The endosperm constitutes the greater part of the grain and consists of numerous cells of net-like form in which the starch granules are tightly packed in an amount averaging 70% of the total weight of the grain.

In estimating the relative value of wheat for brewing purposes the grain should be uniform in size with a fine lustre, light yellow in colour, free from admixture and with plenty of meal. If on biting a berry the fracture appears clear and horny the grain contains too much protein and a low percentage of starch.

Wheat contains an average of 12% protein comprising five different substances, glutenin, gliadin, leucosin, globulin and proteose. The latter is possibly formed during the process of extraction by the modification of other proteins.

Gluten is composed of gliadin and glutenin in approximately equal quantities, the gliadin forms a sticky medium with water which binds together the particles of glutenin to a coherent mass possessed of considerable tenacity and elasticity. In addition to the proteins, wheat also contains certain nitrogenous bases such as asparagine, choline and betaine.

Rye. *Secale Cereale.* Is a hardy grass largely cultivated in Central and Northern Europe and to some extent in America and England. The grain is similar in structure to wheat, but the individual grains are much narrower and darker in colour. The flour, made into bread, is dark coloured and sour, but owing to the liability of rye to engender ergotism it is by no means a safe or wholesome food. The grain is extensively used in the raw state, and as malt, in the distillery and yeast industry.

Rye contains in all an average of 9% protein composed of five substances, albumin, gliadin, glutelin, globulin and proteose. Rye does not form gluten and contains much gummy carbohydrates which renders the isolation of the proteins a matter of difficulty. They are closely related to those of wheat, the portions soluble in water and salt solution being identical in the two cereals.

Barley. *Hordeum Vulgare.* This is the most widely cultivated of all cereals and has been known since the dawn of history. It differs from wheat and rye in that it is closely

invested by its chaff and is therefore classed with the spelts. The ear of barley is a compound spike bearing on two sides spikelets of three flowers each.

The commonest and most valuable variety has two rows of grain, only the central flower in each spikelet ripens, the two lateral ones remaining barren. In the colder parts of Europe the four-rowed type is chiefly grown, all its flowers are fertile, but instead of forming six rows they arrange themselves so as to give the appearance of four. The six-rowed variety with all its flowers fertile is an ancient form, less valuable than the two foregoing.

In the barley corn the aleurone layer consists of four rows of cells instead of the single one in wheat and rye, and, like rye, barley is deficient in gluten, only a very small amount of which can be obtained by washing.

In estimating the value of barley for brewing purposes the grain should be uniform in size, plump and short with a light-textured husk. Small grains contain less starch but more protein, cellulose and ash. A hollow or a shrivelled tip indicates exposure to frost or harvesting before maturity. The moisture content differs according to the ripeness of the grain and the conditions during harvesting and storage. The total protein of barley averages 10·7%, made up of five constituents in the following proportions: glutelin, 4·42%; hordein, 4·04%; globulin, albumin and proteose, 2·24%.

Oats. *Avena Sativa.* Like barley, this grain belongs to the spelts. The husk is very adherent and it is necessary to kiln the oats in order to divest the grain of it.

Oats are used both raw and malted, but only to an insignificant extent, although the large amount of husk makes this grain an excellent aid to filtration. Wort derived from a mash in which an appreciable percentage of oats is included is of a very frothy nature and unsuitable for the turbulently aerated differential fermentations.

The study of the oat proteins is a matter of considerable difficulty and as yet incomplete. The aqueous extract is very acid and the proteins are rapidly hydrolysed by the proteolytic enzymes.

Maize. *Zea Mays.* This is a true grass and is known only

in the cultivated state. The plant grows to a height of 11 feet. It is monœcious, producing beautifully tasselled male flowers and the female flowers, which develop the cobs, are found closely attached to the central axils of the leaves below. Maize is rich in carbohydrates, containing sometimes more than 70% of starch in addition to appreciable quantities of dextrin and sugar. The endosperm consists of a friable portion filled mainly with starch, and a horny portion in which the starch granules are intimately mixed with and cemented by the protein.

An important feature is that the corn has a comparatively large embryo, the oil content of which ranges between 6% and 7%. Maize contains an average of between 8·5% and 9% protein, composed of zein, glutelin, globulin, albumin and proteose, but differs from wheat in producing no gluten.

Tapioca. *Cassava, Manioc.* This is a tropical shrub growing normally to a height of from 5 to 7 feet, but under suitable conditions of soil and climate it may reach as high as 10 feet.

The value of the plant lies in its large tubers which, when freshly harvested, have an average weight of from 8 to 10 lb. and a length of from 18 to 24 inches. Externally the tubers have a dark yellow or brown colour and contain a quantity of poisonous juice, consisting mainly of hydrocyanic acid; this, however, is very volatile and is dissipated in the drying process, during which the moisture content of the tubers is reduced from 65% to 8% or 10%, with a corresponding reduction in the size of the tubers, which then contain from 78% to 86% of starch.

There are two species of cassava—the bitter *Manihot Utilissima* and the sweet *Manihot Aipi*. The most important is *Manihot Utilissima*, or *Jatropha Manihot*, the root of which may attain weights up to nearly 35 lb., and in some cases contains up to 40% starch. In the West Indies and the West Coast of Africa, as well as in South America, this species is an important plant, not only as producing an article of native food, but also the well-known tapioca of commerce. The starch granules of tapioca are readily identified, being far smaller than those of any other starch-producing plant.

In a bulletin issued by the Bureau of Science at Manilla it is stated that 1 acre of cassava yielding 10 tons of roots would give 5,000 lb. of extractable starch in addition to from 4% to 6% of fermentable sugars.

Potato. *Solanum Tuberosum.* The potato is not commonly used in brewing, except in parts of Europe where tubers of a type especially adapted for manufacturing purposes are cultivated. The tubers of the plant consist of a brown skin enclosing a more or less translucent substance of a creamy colour, built up of cells enclosing the starch granules. Water constitutes the great bulk of the potato, the proportion ranging from 60% to 80%, according to the conditions of growth, and in a lesser degree to the type of plant. Like the water content, the percentage of starch is largely influenced by external influences and fluctuates between 10% and 20%, exceeding the higher value only under exceptionally favourable conditions of growth.

Malt. Malt is produced by steeping grain in water until it softens, and inducing it to germinate on a specially prepared floor, at suitable temperatures, for a period depending upon the specific purpose for which the malt is intended. The nourishment of the embryo consists of the starch, protein and mineral matter stored in the endosperm during the vegetative period of the plant.

In the malting process the composition of the grain is materially altered. There is a reduction in the amount of starch consumed by the germ, an increase in the amount of soluble carbohydrates, and an alteration in protein. In germination, enzymes are produced by the embryo to attack the starch and nitrogenous substances, changing them into sugar and amide compounds, which, together with phosphates, constitute the three main substances upon which the germ subsists.

Cytase and diastase are formed in the germ in an amount exceeding that required to satisfy the needs of the growing germ for food and diffuse through the endosperm as growth proceeds. Cytase attacks and dissolves the cellulose membrane in which the starch granule is enveloped, thus facilitating the solution and hydrolysis of the starch granule by the diastase.

Simultaneously the insoluble proteins are dissolved by the proteolytic enzymes and, with other foods, transported to form the structure of the young plant.

The removal of the starch, protein and mineral substances consumed by the growing germ takes place along diminutive passages intersecting the endosperm, which thus becomes porous and spongy by the dissolution of material as growth progresses. When the acrospire of the embryo has developed to between three-quarters and the full length of the grain, germination is interrupted by reducing the moisture in the malt by gentle drying in a current of warm air; the malt is then completely dried in a specially constructed kiln, in which the temperature is carefully controlled to avoid destruction of the diastase by overheating.

Temperature affects the diastatic activity more severely while the malt is moist than after it becomes dry; therefore the modification of the malt that takes place in the kiln depends upon the proper adjustment of the temperature to the degree of moisture in the malt. The more moisture that is expelled before the temperature is raised the greater will the diastatic activity of the malt remain.

The chief object of malting is the production of enzymes, which, by their subsequent action in the mash tun, provide the materials for yeast growth and alcohol production. Thus while the germ is unnecessary in brewing and its consumption of material depletes the endosperm, the development of the enzymes and consequent modification of the grain contents are indispensable for the production of worts. Malt can be made to produce far more diastase than is necessary to convert the malt starch alone to sugar, and is thus able to hydrolyse the gelatinized starch of a considerable quantity of raw grain when mashed with it.

Well-malted barley ranges in colour from light to dark yellow, according to the origin of the grain and the degree of kilning. On breaking the corn the interior should be pure white and friable. It is customary to form an opinion of a sample of malt from its crispness and flavour; each corn should break easily between the teeth and the sweet characteristic malty flavour should be quickly developed.

Glassy corns are caused by insufficient modification or by hastily raising the temperature in the kiln when the malt contains a high percentage of moisture. A properly malted mellow malt will float in water and glassy corns will sink.

During the malting process the proteins of barley are very much changed, hordein disappears and a protein of entirely different composition, known as bynin, takes its place. The globulin is replaced by a new substance called bynedestin, and, although the albumin remains unaltered, it increases in quantity.

Brewing conditions influence the amount of protein dissolved from malt during the mashing process; under normal conditions the percentage increases with the temperature—at first uniformly up to 38° C. and then more rapidly to the maximum at 49° C. when it amounts to 40% of the total nitrogen of the malt. The amount dissolved declines somewhat from 49° to 60° C., and still more up to 62·5° C., the saccharifying temperature of the mash.

Malt Culms. In drying and kilning malted grains the withered sprouts and rootlets, known as culms or culmings, are easily separated from the grains in the cleaning process, and, owing to their exceptional food value, find a ready sale to cattle-feeders and yeast factories. The culms not only contain a very high percentage of assimilable nitrogen, mostly in the form of asparagin, but their bulky fibrous nature aids mash filtration in such a marked degree that culms are an almost indispensable ingredient of the mash in producing worts for yeast cultivation.

They are produced in an amount averaging 3·0% of the weight of the malted grain and normally contain 10·0% moisture, 28·0% protein, 2·0% fat, 39·0% nitrogen-free extract, 14·0% fibre and 7·0% ash, but they contain very little fermentable carbohydrate matter and yield about 600 c.c. of alcohol from 100 kg. of culms.

For brewing purposes they should be pale-straw or cream in colour, long, curly, and free from dust and grit. An exceptionally high percentage of the protein of good quality culms is soluble and readily hydrolysed to diffusible modifications in the mash tun. The culms separated from colour malts or

any malted grain dried at high kiln temperatures are of little value owing to the destruction of proteins by the temperatures employed and the dark colour imparted to the wort by caramelized matter extracted from the burnt culms is objectionable.

Their extract value is determined by means of a simple mashing process: 25 gm. of the culms are weighed, mashed with 400 c.c. of water at 144° F. for one hour on the water-bath, cooled to 60° F., made up to 450 gm. with water at the same temperature and filtered through a ribbed filter paper. The specific gravity of the filtrate is determined and the extract expressed in degrees Balling by referring to the table of specific gravity and degrees Balling equivalents.

Culms of good quality are able to absorb and retain a very large amount of moisture, increasing in bulk in the process to form a loose spongy mass. The absorptive power decreases with the quality, and this feature serves to determine the comparative filter value of the product; 25 gm. of the sample are weighed and mashed in a beaker with 200 c.c. of water at 158° F. for fifteen minutes with occasional stirring; 1 gm. of cotton-wool is placed in the apex of a funnel and the mash filtered through this into a graduated cylinder. The volume of the filtrate is noted after ten, thirty and sixty minutes and compared with the readings obtained from the examination of a standard sample.

Sugars. The carbohydrates are divided according to their composition into four distinct groups. The monosaccharides, $C_6H_{12}O_6$, are the simplest of the series, and all other carbohydrates can be hydrolysed to two or more molecules of monosaccharide by means of acids or enzymes. They contain from three to eight carbon atoms, and are known as trioses, tetroses, pentoses, hexoses, etc., according to the number of carbon atoms within the molecule. Only the hexoses and trioses are fermented by yeasts; the higher and lower members of the group are industrially unimportant.

Sugars of the disaccharide group have the formula $C_{12}H_{22}O_{11}$, and include such important types as maltose and cane sugar. Trisaccharides consist of sugars of the formula $C_{18}H_{32}O_{16}$. The polysaccharide group embraces compounds of high

molecular weight of the general formula n $C_6H_{10}O_5$, such as starch and cellulose.

Monosaccharides

Dextrose (glucose, grape sugar or starch sugar) is produced from various polysaccharides by hydrolysis, and is found ready formed in many plants, fruits and honey. It crystallizes in white rounded masses containing one molecule of water of crystallization. The crystals remain unchanged in the air, but melt when heated on the water-bath, and at 100° C. lose their water of crystallization. Dextrose dissolves in its own weight of water at 17° C. It reduces Fehling's solution, is about one-third as sweet as cane sugar and is fermented directly by yeast.

Lævulose (fructose, fruit sugar) is found associated with dextrose in most sweet fruits and honey, and a small amount occurs in malt. It closely resembles dextrose, but is very soluble in water and alcohol and crystallizes with considerable difficulty from cold absolute alcohol. Lævulose is much sweeter, but ferments more slowly than dextrose. It reduces Fehling's solution, and when heated to 170° C. loses one molecule of water and is converted to levulosan. By gentle heating with dilute acids, cane sugar is transformed into the mixture of almost equal parts of dextrose and lævulose known as invert sugar.

Galactose is produced with dextrose by boiling lactose with dilute acids. It is soluble in water, crystallizes in hard rhombic prisms that are less sweet than cane sugar and melt at 163° C. It resembles dextrose in many of its reactions.

Disaccharides

Maltose is the disaccharide formed as the product of the hydrolysis of starch by the enzyme diastase. It crystallizes in masses composed of hard white needles and loses water of crystallization when heated to 100° C. A solution of maltose reduces Fehling's solution. It is hydrolysed to two molecules of dextrose when heated with dilute acids, but the trans-

formation is more rapid under the influence of the enzyme maltase. In the hydrolysis of starch by enzymes about 80% is converted into maltose, the remaining 20% being stable dextrin.

Saccharose (sucrose, cane sugar) is found in the juice of the sugar cane, the sugar beet, and in the sap, seeds and fruit of many other plants. It forms large transparent crystals, the specific gravity of which varies between 1·55 and 1·61, according to the mode of crystallization. It is soluble in boiling water in all proportions and in less than half its weight of cold water, a concentrated solution holding about 66% sugar.

When cautiously heated it melts at about 160° C. and decomposes above this point. When subjected to prolonged boiling, saccharose acquires an acid reaction and becomes partly inverted. It is not directly fermentable, but when heated with dilute acids it changes into the fermentable invert sugar, consisting of dextrose and lævulose. This hydrolysis is also effected by the enzyme invertase found in yeast.

Lactose is only met with in products derived from milk, and is obtained as hard colourless rhombic crystals that dissolve in 6 parts of cold water and in 2 parts of boiling water. It has a faint sweet taste and is hydrolysed by acids and the specific enzyme lactase into a molecule of dextrose and one of galactose. It is not fermented by yeast, but is changed to lactic acid by certain types of lactic acid bacteria.

Isomaltose is formed by the action of hydrochloric acid on dextrose and in small amount from starch in the presence of diastase. It has an intensely sweet taste, is decomposed by gentle heating and is unfermentable.

Trisaccharides

Raffinose (melitose) is a well-known representative of this group, found in small amounts in barley and wheat during germination, and in larger amounts in sugar beets, but as it is more soluble than saccharose it accumulates in the molasses. It is hydrolysed to dextrose, lævulose and galactose by heating with dilute acids, or in contact with enzymes, and is therefore fermentable. It crystallizes in fine needles that lose water of crystallization at 100° C. It is only slightly sweet, dissolves

freely in water and in alcohol, but does not reduce Fehling's solution.

Polysaccharides

Dextrins. Starch and its decomposition products are members of the polysaccharide group of great interest to the brewing industry. Dextrins are polysaccharides formed by the partial hydrolysis of starch ; they are insoluble in alcohol, but dissolve in water to form a clear solution, and, with the exception of amylodextrin, reduce Fehling's solution, although the reducing power of the higher dextrins is very slight.

There is no ready method of distinguishing the different varieties with certainty as they are separated as individuals with considerable difficulty. Amylodextrin gives a pure blue colour with iodine, Erythrodextrin I. is coloured purple, Erythrodextrin II. gives a red colour, Erythrodextrin III. is coloured red-brown, and Achroodextrin gives no colour at all with iodine. Maltodextrin, the lowest member of the series, does not ferment with most yeasts, but is rapidly hydrolysed to maltose with diastase and to dextrose with acids.

CHAPTER II

THE ISOLATION OF YEAST PURE CULTURES

Preparation of Nutrient Media

MICRO-ORGANISMS are invariably studied in pure cultures grown in solutions containing the proper kinds and amounts of food, or on solid media prepared from such solutions by the addition of gelatine or agar-agar. The food solution may be composed of broth, wort, or fruit juices according to the requirements of the micro-organisms under examination, and in some cases artificial media are devised for special investigations.

Yeast is usually cultivated in sterile nutrient solutions prepared by extracting crushed malt with water at suitable temperatures, as this material supplies the organic and mineral substances in the proportions required for the growth of yeast. The process generally adopted follows the ordinary mashing methods in miniature, supplemented by a process of clarifying and sterilizing the solutions. Any one of a number of methods of producing the food solution may be adopted, but as the properties of yeast vary more or less with variations in their nutriment, uniform results are more certain if a standard procedure is always followed.

Sterile wort is produced by mashing 25 lb. of crushed barley malt with 8 gallons of water at 128° F. in a steam-jacketed pan and raising the temperature of the agitated mash to 145° F. for sixty minutes' saccharification. The wort is filtered from the grains, returned to the pan, boiled, 'fined' by the addition of egg-white, isinglass or Irish moss, and allowed to clarify by sedimentation. After cooling, the clear wort is syphoned off the sediment into a number of 2-litre flasks, which are then closed with cotton-wool plugs and the contents sterilized by boiling. On cooling a further precipitation occurs and the wort

is filtered bright through paper pulp, prepared by moistening sheets of filter paper with wort and stirring until the paper is disintegrated.

The apex of a 9-inch funnel is loosely plugged with cotton wool and filled with the wort-pulp mixture, from which the filtrate runs bright and clear, the pulp forming a pad which retains the finest particles. The filtered wort is placed in flasks in amounts depending upon the purpose for which it is required. Seed-yeast cultures, for instance, are grown in volumes of wort that increase progressively as the culture is transferred from one generation to another, commencing with 50 c.c. and increasing to 250 c.c., then 1,000 c.c. and 2,000 c.c., and finally into 4,000 c.c., which marks the final stage of the laboratory cultures. Volumes of 1,000 c.c. and over are usually contained in 2-litre flasks, each holding no more than 1,000 c.c.

The brightly filtered wort is diluted to 12° Balling and divided into 50 c.c. portions in a number of 100 c.c. Frudenrich flasks through a 3-inch funnel, carefully avoiding contact of the wort with the necks of the flasks, which are then immediately closed with the glass caps, the gas escape tubes of which are closed with cotton-wool. Erlenmeyer flasks of 500 c.c. capacity are charged with 250 c.c. of the diluted wort, observing the same precautions to avoid contact of the wort with the neck of the flask, which would encourage infection and cause trouble with the cotton-wool closure in the process of inoculation.

The flasks are closed with cotton-wool plugs and these are protected from dust by capping with discs of greaseproof paper. The correct size of the cotton-wool closures may be determined by lifting the flask by the plug ; if it will not sustain the weight of the flask the plug is too loose, and a plug that is too tight causes trouble and delay when opening the flask for inoculation. The contents are then sterilized by placing the flasks in the steam sterilizer for fifteen minutes each on three successive days.

The remainder of the 12° Balling wort is filled into a number of 2-litre Erlenmeyer flasks in volumes not exceeding 1,000 c.c. and, after closing with cotton-wool and capping with greaseproof paper, are sterilized by boiling on the sand bath for ten to fifteen minutes on three successive days.

Sterile grain mash is used for the cultivation of lactic acid bacteria and also to restore the virility of yeast degenerated by repeated growth in acid worts, as a preliminary to the isolation of pure cultures from commercial yeasts.

A mixture of equal parts of crushed barley and wheat malts, totalling 400 grams, is mashed with 1,650 c.c. of water at 125° F. and heated to 145° F. for sixty minutes' saccharification. The coarse particles are removed by straining through a hair sieve and the liquid filled into conical flasks of suitable capacity, closed with cotton-wool and covered with dustproof caps.

The flasks are fractionally sterilized on three successive days, and after standing at room temperature for three days to confirm freedom from infection, the mash is again sterilized by boiling on the sand bath for ten minutes.

Solid Transparent Media. Gelatine or agar-agar is used in the preparation of solid media for Petri-dish cultures, the growth of giant yeast colonies, the preservation of pure cultures, and as a convenient medium for the examination of all kinds of yeasts, moulds, bacteria and protozoa. Both of the substances are innutritious alone and are used as agents to solidify the natural and artificial food solutions suited to the particular needs of the micro-organisms under examination.

Wort-agar is prepared by dissolving agar-agar, at the rate of 2% by weight, in 12° Balling wort followed by filtration and sterilization. Wort-agar liquefies at 194° F. and congeals at 104° F.

The agar is cut in small pieces and after addition to the wort it is allowed to stand for several minutes to saturate and swell. It is then placed on the water bath, or in the steam sterilizer, for several hours until dissolved ; the solution is then filtered.

Owing to the readiness with which the wort-agar solidifies the filtration process is very difficult, and for this reason the solution is filtered either in a steam chest or by means of a hot-water funnel, passing small quantities only through the filter at a time with frequent changes of the filter paper. Cotton-wool may be used instead of filter paper if a transparent medium is not essential, or filtering may be dispensed with entirely by simply decanting the clear portion of the hot

solution after sedimentation. Wort-agar is seldom perfectly transparent and a little opaqueness is permissible. The wort agar is filled, in 20 c.c. portions, into 100 c.c. Freudenrich flasks and fractionally sterilized by heating in the steam sterilizer. Water of condensation usually separates out and in cooling is deposited on the surface of the solidified medium. To avoid a spreading yeast culture, due to this water, it is advisable to allow the moisture several days to dry out before use.

Wort Gelatine. Sterile wort at 12° Balling is heated to 145° F. and gelatine is added in an amount corresponding to 10% of the weight of the wort. The flask is gently heated on the hot-water bath until all the gelatine is dissolved. The mixture is then filtered through cotton-wool, or through filter paper if a transparent medium is desired. Filtration is only possible while the medium is hot as the gelatine coagulates at temperatures below 95° F. and prevents further filtration. Wort gelatine should not be boiled, and if acid worts are used the gelatine may not set. The filtered wort gelatine is liquefied and filled, in 20 c.c. volumes, into 100 c.c. Erlenmeyer flasks and fractionally sterilized in the steam sterilizer.

Bouillon is a cooked infusion of beef, made neutral or slightly alkaline with sodium carbonate, used principally for the cultivation of bacteria and only occasionally for some special examination of yeast; 500 gm. of finely chopped raw lean beef are placed in a flask, covered with 1 litre of cold distilled water and left standing twelve hours with occasional stirring. It is then strained through clean white muslin and gently pressed until 1 litre of liquid is obtained.

After the addition of 10 parts of peptone powder and 5 parts of common salt, the solution is boiled, cooled, carefully neutralized to litmus paper by the addition of sodium carbonate solution and filtered. The broth is filled into flasks in quantities depending upon the object of the investigation and fractionally sterilized in the steam sterilizer.

Yeast Water contains nutriment in a form readily assimilated by most micro-organisms, but it requires the addition of alcohol for the cultivation of mycoderma and acetic acid bacteria, and sugar for investigating yeast or lactic acid bacteria. It is prepared by boiling 100 gm. of compressed yeast in 1 litre of

water and pouring the solution into a cylinder to cool and deposit the yeast. The clear liquid is decanted, filtered through filter paper and, after the addition of any special ingredient required, fractionally sterilized in the steam chest.

Artificial Media. Artificial nutrient media were first introduced by Pasteur during his memorable controversy with Liebig, to demonstrate certain properties of yeast; their value was immediately appreciated and artificial food solutions came into general use for many special bacteriological investigations. Owing to the diversity of material consumed as food by micro-organisms a normal, or universal, solution is not possible; the composition of the medium must be carefully adapted to the needs of the subject under examination.

Artificial Food Solutions for Yeast.

Dextrin and sugar	100 grams	$MgSO_4$ . . .	0·1 gram		
$(NH_4)_2SO_4$ . .	4·75 grams	$CaSO_3$ . . .	0·1 ,,		
KH_2PO_4 . .	0·75 ,,	Water to one litre.			
Sugar . .	15·0%	KH_2PO_4 .	0·2%	$CaCO_3$.	0·1%
Water . .	84·0%	$MgSO_4$.	0·1%	$(NH_4)K_2PO_4$	0·5%
Sugar . .	15·0%	Water .	83·5%	$MgSO_4$.	0·2%
Asparagin .	0·7%	KH_2PO_4 .	0·5%	$CaCO_3$.	0·1%
Sugar . .	15·0%	Water .	83·5%	$MgSO_4$.	0·1%
Pepton Witte	0·5%	KH_2PO_4 .	0·5%	$CaCO_3$.	0·1%

Artificial Food Solutions for Lactic Acid Bacteria.

Pepton Witte	0·5%	Asparagin.	0·3%	Water .	93·5%
Dextrose .	5·0%	KH_2PO_4 .	0·5%	$MgSO_4$.	0·2%

Sugar . 5·0% Extract from 5·0% yeast. Water to 100 parts.

Artificial Food Solutions for Acetic Acid Bacteria.

Meat Extract	2·0%	Dextrose .	2·5%	Alcohol .	4·0%
Pepton Witte	0·5%	Water .	91·0%		
$(NH_4)_2PO_4$.	0·1%	KH_2PO_4 .	0·2%	Alcohol .	5·0%
Cane Sugar .	3·0%	Water .	91·5%	$MgSO_4$.	0·2%

The Isolation of Yeast Pure Cultures

The growth of a culture propagated from a single cell in sterile nutrient medium, effectively protected against outside

infection, are essential features of any rational process for the production of pure cultures. There are several methods of isolating the single cells ; in liquid medium this is accomplished by diluting the mixed growth with sterile liquid medium until each drop of the solution, when transferred to a glass slide, contains only one cell. In solid transparent medium the isolation is effected by diluting the mixed growth with wort agar until each cell is widely separated from its neighbour when the solution is spread in a thin layer upon suitable plates, so that the type and shape of the cells, the development of the colonies, or their contamination with bacteria or moulds, may be observed by microscopic examination.

The suitability of any particular yeast colony, or culture, for commercial purposes depends not so much upon its microscopic appearance, which does not always reveal degeneration even in routine cultures, as upon physiological properties that can only be determined in large amounts of yeast obtained from the later development of the cultures, but the type of yeast cultivated for the baking trade exhibits characteristic features during the early stages of its laboratory cultivation that indicates its suitability for rapid filter-pressing in the final stages of the process. It forms a compact sediment on the bottom of the flask that is difficult to disturb, and when the flask is shaken or rotated a portion of the sedimentary yeast rises in smooth streamlines without the slightest indication of flocculation ; a quality that is attributed to the physical condition of the cells, which are smooth like starch granules and devoid of the glutinous envelope typical of flocky or slimy yeast.

Yeast readily adapts itself to its environment, but, unfortunately in some cases, its characteristics are also modified with variations in its cultural conditions. Nearly every yeast factory develops a type of its own, differing from others in its fermenting and attenuating power. Differences in the raw material and its treatment contribute to these changes, but in a far less degree than the influence of variations in pure culture methods upon the young cultures. A standardized pure culture routine is essential, and any proposed alteration in method is carefully investigated to determine its possible influence upon the properties of the commercial yeast before final adoption.

In the preparation of hanging-drop cultures the interior of the sterile chamber (Fig. 1) is first sprayed with a solution

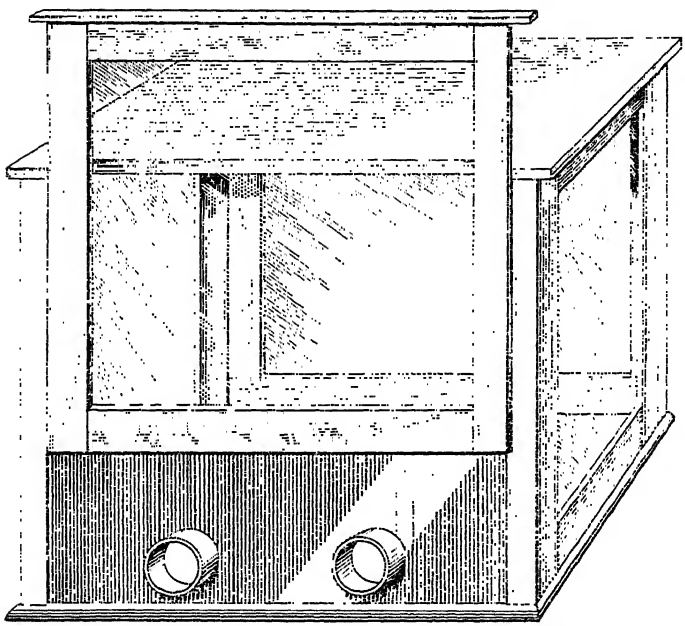


FIG. 1. Bacteriological sterile chamber in which pure cultures are isolated and cultures are transferred from one flask to another.

containing 1 part of mercuric chloride in 1,000 parts of water and allowed to stand for thirty minutes. In all pure-culture work the cultures are transferred or exposed only under conditions that ensure protection against outside contamination, and, as a matter of safety, the platinum needles, flasks and all small apparatus, are habitually passed through the flame before and after each procedure.

With a long platinum needle (Fig. 2), the end of which is bent into a loop to which a globule of liquid will adhere, 1 drop

FIG. 2. Platinum needle and loop used for transferring cultures, made by fusing platinum wire in glass rod to form a holder.

of the mixed yeast growth is transferred to a flask containing 50 c.c. of sterile wort, then gently shaken to distribute the cells evenly in the medium.

A microscope glass slide with a circular cavity in the centre and a square cover glass are sterilized in the flame, placed in the sterile chamber, and, after cooling, the edge of the cavity is smeared with a ring of vaseline, on which the cover glass is lightly placed to obtain an impression of the vaseline ring; it is then lifted with the thumb and middle finger at opposite corners while the index finger supports its back. A pointed platinum needle is dipped in the inoculated sterile wort and one or more rows of minute drops are made on the cover glass inside the vaseline ring. After breathing into the cavity to supply water vapour, the cover glass is replaced in its original position on the slide and gently pressed until sealed by the vaseline to form a sterile airtight cell, from which the drops cannot evaporate.

The prepared slide is then microscopically examined, and the drops containing a single yeast cell only are noted on a diagram drawn on a piece of adhesive paper attached to the end of the slide. If all the drops contain more than one cell greater dilution is necessary, and the procedure is repeated until the correct dilution is obtained. The slide is then placed in the incubator at 84° F., and after twenty-four hours it will be found that while all the drops show an increase in the number of cells the vigorous cultures have increased by hundreds.

The selected drop is transferred on the point of a platinum needle to a flask containing 50 c.c. of sterile wort and incubated at 84° F. until the culture develops sufficient yeast for further investigation and analysis of the fermented wort.

In the routine isolation of pure cultures for seed-yeast production the method of Petri is generally adopted, using two shallow glass dishes, one inverted to cover the other, and transparent solid media. The Petri dishes are placed in cold water, heated to boiling, and, after wrapping in grease-proof paper, sterilized in a hot-air oven at 300° F. for fifteen minutes The dishes are then transferred to the sterile chamber, and after cooling removed from the paper cover.

The cells of a mixed yeast growth are isolated by one or more dilutions in sterile wort until the requisite dilution is obtained, and then in liquefied wort-agar, which, on cooling and solidifying, imprisons every cell, each of which on growing produces a colony. A small percentage of these colonies may develop from two adhering cells, such as mother and daughter cells, and thus fail to be a pure culture.

An Erlenmeyer flask containing 20 c.c. of sterile wort-agar is immersed in boiling water until the medium is liquefied, the cotton-wool plug is singed to free it from dust, and the flask is placed in a beaker or small water-bath containing a thermometer and about 75 c.c. of water at 120° F.

One drop of the liquid containing the mixed yeast is transferred on the loop of a platinum needle to 25 c.c. of sterile wort contained in a 100 c.c. flask and gently rotated to keep the cells in suspension. This is termed the first dilution.

When the thermometer immersed in the water-bath indicates 105° F. the flask of wort-agar is quickly conveyed to the sterile chamber, the stopper is removed and a drop of wort transferred on the loop of a platinum needle from the first dilution flask to the fluid wort-agar. This is gently rotated to distribute the cells and the flask immediately emptied into the bottom portion of the Petri dish, tilting slightly as required to cover the bottom with a uniform thickness of film. The culture is immediately covered with the top portion of the dish and allowed to stand until the wort-agar solidifies. The operation of inoculating and pouring the wort-agar into the Petri dish must be rapidly conducted as wort-agar sets between 104° and 105° F., and higher temperatures cannot be used without injury to the yeast.

Water of condensation may separate from the warm wort-agar, and, after condensing on the cover, run back and spoil the culture as soon as the dish is moved. Porous covers were introduced to overcome this defect, but the transparent tops are preferred and condensation avoided by placing the warm culture over sulphuric acid in a desiccator for a few hours until the separated moisture is absorbed.

After three days' incubation at 80° F. each cell develops a visible colony, and when the dilution is correct, between thirty and forty colonies will be isolated and evenly distributed over the surface of the medium. Twenty or more of the genuine yeast colonies are selected and each one transferred, on the point of a platinum needle, from the Petri dish to a separate flask containing 20 c.c. of sterile wort.

The twenty flasks are incubated at 84° F. for three days and each culture is subjected to a critical microscopic examination, discarding any that show infection or abnormality of any description, the remaining cultures are transferred to 50 c.c. of sterile wort contained in 200 c.c. flasks, and after two days' incubation at 84° F. the sandy nature of the sedimentary yeast in each flask is confirmed, and any culture that exhibits the slightest indication of flocculation is rejected. The cultures passing this test are mixed together in a 2 litre flask, and from this " multiple culture " the permanent culture in pure sugar solution, and the giant colonies on wort-agar are prepared.

The isolation of cultures is not essential for the commencement of every seed-yeast culture ; unnecessary repetition of this work is avoided by the inoculation of a number of flasks of sterile wort-agar with the yeast from the multiple culture and incubation at room temperature until a giant colony about 1 cm. in diameter is formed on the surface of the solid medium in each flask.

These cultures deteriorate when exposed at room temperature for extended periods, but at temperatures not exceeding 40° F. the vitality of the yeast is fully preserved, and the cultures may be used as the starting-point for seed-yeast cultures for periods up to three months after their inoculation. Degeneration of the colonies commences with the shrinkage of the wort-agar and discoloration of the edges of the growth, but before this occurs the cultures are " cleaned " or regenerated by the isolation of twenty cells and their development into cultures as described.

Giant colonies are produced by inoculating sterile wort-agar, contained in Freudenrich flasks. The surface of the solid medium is lightly touched with the point of a looped platinum

needle previously moistened by dipping it into the multiple culture. Growth proceeds at room temperature for seven days, or until the disc-shaped colonies measure about 1 cm. in diameter. The flasks are then marked with the date and identification number and kept at temperatures not exceeding 40° F.

The preservation of the particular race of yeast employed is a matter of utmost importance in every yeast factory, and to ensure a reserve in the case of mishap, or failure in isolating colonies, a permanent culture in cane sugar solution is inoculated from each multiple culture. The sugar solution is prepared by dissolving 10 gm. of pure nitrogen-free cane sugar in 100 c.c. of freshly distilled water, the solution is then boiled, and, after division into 20 c.c. portions in Freudenrich flasks, fractionally sterilized in the steam chest. After cooling, the contents of one or more of these flasks is inoculated by transferring a minute drop of the multiple culture, on the loop of a platinum needle, to the sugar solution; the flask is closed, and, after marking with the date and identification number, immediately placed in cool store. The vitality and general characteristics of the yeast are undiminished after ten years' preservation in this solution, which may at any time be used directly as the starting-point for a seed-yeast culture.

A position may occur when it becomes necessary to isolate cells and produce seed-yeast cultures from commercial yeasts, but the repeated growth in acid wort, essential in the production of commercial yeast, renders it quite unsuitable for the direct isolation of pure cultures intended for seed yeast, and satisfactory results are obtained only when this yeast is regenerated by vigorous preliminary fermentations in sterile grain mash.

A flask containing 100 c.c. of sterile grain mash is inoculated with 10 gm. of the commercial yeast under examination and incubated at 80° F. for twelve hours. The fermented mash is then transferred to a flask containing 1 litre of sterile mash, and, in addition to this large seeding, the activity of the yeast is further stimulated by incubation at 84° F.

The energetic fermentation thus induced serves a double purpose: (1) the yeast is completely regenerated; and

(2) the growth of foreign micro-organisms retarded to such a degree that it is exceptional to find any in the cultures which follow. After twenty-four hours' fermentation the yeast cells are microscopically examined and usually found quite spherical in shape with transparent plasma and a large clearly defined vacuole. A Petri dish culture is then prepared from the rejuvenated yeast and twenty of the most vigorous colonies selected and cultivated as previously directed.

A record of the isolation of pure cultures, or the cleaning of giant colonies, is usually detailed in a book kept for the purpose in columns with the following headings :—

PURE CULTURE RECORD

RACE. TYPE. ORIGIN

Date Plated.	Plated by.	Colonies selected.	Colonies used.	Date Giant Colonies Inoculated.	Permanent Cultures Inoculated.	Culture Number.

Seed Yeast and Its Production

The quantity of seed yeast used to inoculate, or seed, a brew is expressed as a percentage of the weight of raw material used in the mash, and may vary between 2% and 15%, according to the product required. For alcohol production the smaller percentages are generally used, but for the cultivation of commercial yeast in differential brews the conditions are arranged and control directed to stimulate cell reproduction to the utmost, and as the number of new cells formed is directly proportional to the number of mother cells present, the amount of seed yeast that may be used is limited only by the available nutriment.

Under normal conditions each yeast cell is able to decompose a definite amount of sugar in a given time, consequently the rate of fermentation is proportional to the amount of yeast present in the fermenting solution. Variations occur however when the vitality of the seed yeast is impaired by high temperatures in filter-pressing or in storage, which may reduce both its reproductive and fermentative activity. The age of seed yeast is immaterial provided that it is separated and pressed at low temperature and stored in a dormant condition at 32° F.

without freezing, but as the latter condition is somewhat difficult to maintain for lengthy periods it is customary to produce a seed-yeast brew every fourteen days and to keep the yeast in cool store at temperatures not exceeding 40° F.

In former methods of seed-yeast cultivation the amount of yeast transferred from one culture to another was very small, the growth from the agar colony to 5,000 gallons of wort was effected in from five to six transfers, or generations, consequently a far greater volume of wort was used at each transfer, and as yeast growth often ceased before all the food was consumed the development of attenuating power was quite neglected.

One of the principles of the differential process requires sufficient yeast in each transfer to stimulate competition between the individual cells for the available nutriment; and in the energetic fermentation that follows, the wort is attenuated to a degree not otherwise possible. In addition, dextrins are attacked and accidental infection eliminated or suppressed.

With such large seedings the fermentation is vigorous, and if the temperatures were not controlled the heat developed would be injurious to the yeast, fermentative activity is therefore retarded by incubating the laboratory cultures at 80° F., and the smaller factory cultures at temperatures between 72° F. and 80° F. by means of special cooling apparatus suited to the vessels and tanks in which the generations are fermented.

The following brief outline illustrates the number and volume of the generations that succeed one another during the twelve days occupied in cultivating yeast from the agar colony to the final generation in which ten brews of commercial yeast are produced. In earlier methods it was possible to vary the size of the seed-yeast brew to suit the needs of the moment, but in this process the progressive generations bear a definite relation to each other and the elimination of any one transfer, or difference in the amount of yeast produced, may seriously interfere with the production routine, a feature that becomes more apparent as the culture approaches the final stages of the process.

Generation.

1. 20 yeast cells in 50 c.c. of sterile wort.
2. (50) + 200 c.c. of sterile wort.
3. (250) + 1,000 c.c. of sterile wort.
4. (1,250) + 4,000 c.c. of sterile wort. (Decant surplus liquor.)

Laboratory cultures fermented in glass flasks at 84 ° F.

5. (4) + 12 litres of acid wort = 16 litres. Ferments 24 hours between 73·5 and 80 ° F.
6. (16) + 48 litres of acid wort = 64 litres. Ferments 24 hours, 75 to 80° F.
7. (64) + 192 litres of acid wort = 256 litres. Ferments twenty-four hours from 75 to 80° F.
8. (256) + 768 litres of acid wort = 1024 litres + 250 c.c. NH_4OH. Ferments twelve hours from 75 to 80° F. Alcohol 4–5% in fermented wash.

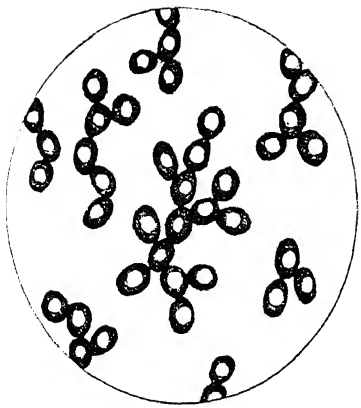
Factory cultures in 12 deg. Balling wort. Acid varies from 4 to 5 deg. Not aerated.

9. (1,024) + 3,072 litres = 4,096 litres at 9° Balling. Acid 4° + 1 litre NH_4OH. Ferments eleven hours between 77° and 82° F. Aerated brew. Alcohol in wash 2·8–3·2 by weight.
10. 1,371 kilograms of raw material produce 16,452 litres of wort. Add $(NH_4)_2SO_4$ then ninth culture. Aerated brew ferments in twelve hours. Alcohol in wash 1–1·2%.
11. One G10 brew yields sufficient yeast to seed two G11 differential seed yeast brews, each produced from 1,371 kgs. of raw material.
12. Two G11 brews yield sufficient yeast to seed ten G12 differential commercial yeast brews, each producing a minimum of 20 cwts. of yeast.

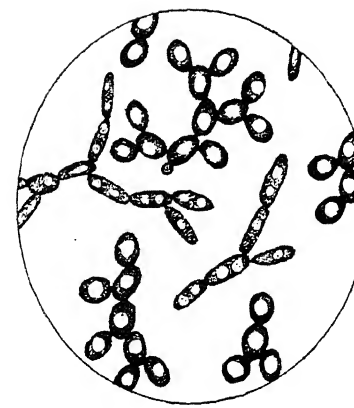
It will be observed that the volume increase of each generation of the factory cultures, including G10, amounts to three times the volume of the preceding generation.

If more commercial yeast is required than that produced by the example brew the factory output is increased by increasing the volume ratio between the successive cultures. With a three-volume increase to each generation, 452 kg. of G10 yeast are produced and this results in the production of 10,282 kilos of commercial yeast. A four-volume ratio between each transfer produces 1,687 kilos of G10 yeast and 42,180 kilos of commercial yeast. Thus any output between these quantities may be produced by a fractional variation in the volume increase of the progressive cultures.

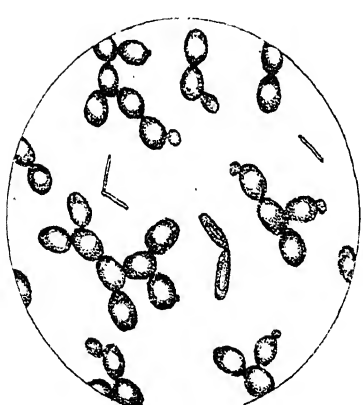
Eleven days before the commercial yeast is required the



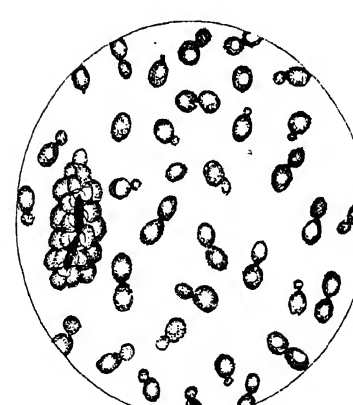
A. The typical appearance of yeast cells from a pure culture.



B. Saccharomyces Cerevisiæ and Mycoderma Cerevisiæ.



C. Yeast from a spirit fermentation infected with wild yeast and lactic acid bacteria.



D. Pelleted yeast from an infected G12 differential fermentation.

FIG. 3. Original drawings from the microscopic examination of yeast.

[*To face p.* 50.

the incubator without disturbing the yeast sediment and about 50% of the clear fermented liquor is decanted. The cotton-wool plug is replaced and the flask gently rotated to distribute the cells uniformly in the remaining liquid. This is microscopically examined, divided between four flasks, each of 2 litres capacity containing 1,000 c.c. of sterile wort and incubated at 84° F. for twenty-four hours. To minimize the risk of infection precautions are always taken to ensure that the laboratory cultures are exposed only in the sterile chamber, and when transferring the cultures from one flask to another the cotton-wool plug is not touched with the hands, the band or cord securing the paper cap is loosened and the plug removed inside the cap and replaced in the same manner.

When commencing a seed-yeast culture it is necessary to prepare a time-table showing when and on what days the cultures are to be transferred from one vessel to another, and to show the day on which it is necessary to increase the amount of raw material mashed in the routine brew to provide the extra 768 litres of wort for the G8 culture and 3,092 litres for the G9 culture.

SEED-YEAST CULTURE ROUTINE

Generation.	Sterile Wort.	Total Wort.	Start.	Finish.	Days.	Brew 617 kgs. extra raw material.
1	50 c.c.	50 c.c.	Mon. 3 p.m.	Wed. 3 p.m.	2	—
2	200 c.c.	250 c.c.	Wed. ,,	Thur. ,,	1	—
3	1 litre	1,250 c.c.	Thur. ,,	Fri. ,,	1	—
4	4 ,,	5,250 c.c.	Fri. ,,	Sat. ,,	1	—
5	12 ,,	16 litre	Sat. ,,	Sun. ,,	1	—
6	48 ,,	64 ,,	Sun. ,,	Mon. ,,	1	—
7	192 ,,	256 ,,	Mon. ,,	Tues. ,,	1	—
8	768 ,,	1,024 ,,	Tues. ,,	Wed. 3 a.m.	12 hr.	Monday
9	3,072 ,,	4,096 ,,	Wed. 3 a.m.	Wed. 3 p.m.	12 ,,	—
10	Brew	16,452 ,,	Wed. 3 p.m.	Thur. 3 a.m.	12 ,,	—
11	Brew	19,194 ,,	Thur. ,,	Fri. 5 a.m.	14 ,,	—

The containers in which the factory cultures, G5, G6 and G7, are fermented are constructed of sheet copper and very much to the same pattern, increasing in size according to the volume of the culture. They are straight-sided cylindrical vessels, with

a dished bottom that facilitates draining, and a loose cover that fits 3 inches down the outside of the vessel. The edge of the cover consists of a circle of $\frac{1}{2}$-inch copper tubing, perforated with $\frac{1}{16}$-inch holes at $\frac{1}{2}$-inch pitch directed to the side of the vessel. One end of this tube is plugged and the other terminates in a hose union so that a stream of cold water may be sprayed on the sides of the vessel.

Owing to the large amount of yeast transferred in each generation of the factory cultures it is necessary to retard fermentative activity by temperature control. If this fails, overheating may occur with the production of decadent yeast in the plasma of which irregular-shaped granules appear. Yeast in this condition is useless and cannot be regenerated. Granulation is a characteristic feature of the exhausted yeast produced by a high temperature fermentation.

G5 Culture. The G5 container has a capacity of 29 litres and the following dimensions : Diameter, 30·48 cm. (12 inches) ; depth, 40·64 cm. (16 inches). Two rigid handles riveted to opposite sides assist in handling the vessel, which is carefully tinned inside to leave a perfectly smooth surface. Before use the vessel is scoured with hot water, steam sterilized for thirty minutes, the condensed water drained out without removing the cover, and, while still hot, filled to the 12-litre mark with hot wort at 12° Balling direct from the filter tank. The acidity and gravity Balling is determined in a sample taken from the filter tank to avoid exposing that in the G5 container to the risk of infection. The wort is cooled by connecting a cold water hose to the perforated edge of the cover and spraying the sides of the vessel until the temperature of the wort is reduced to 73·5° F. (23° C.).

The contents of the four flasks constituting the G4 culture are agitated, and, after microscopic examination, added to the G5 container with as little exposure as possible, the fermentation then proceeds without inspection for about six hours. The temperature rises steadily to 80° F. in about eight hours and is maintained at that degree until fermentation is finished. Owing to the small volume of this culture the temperature may be controlled by simply immersing the vessel in a water-bath, such as a wooden tub, or other suitable vessel, containing

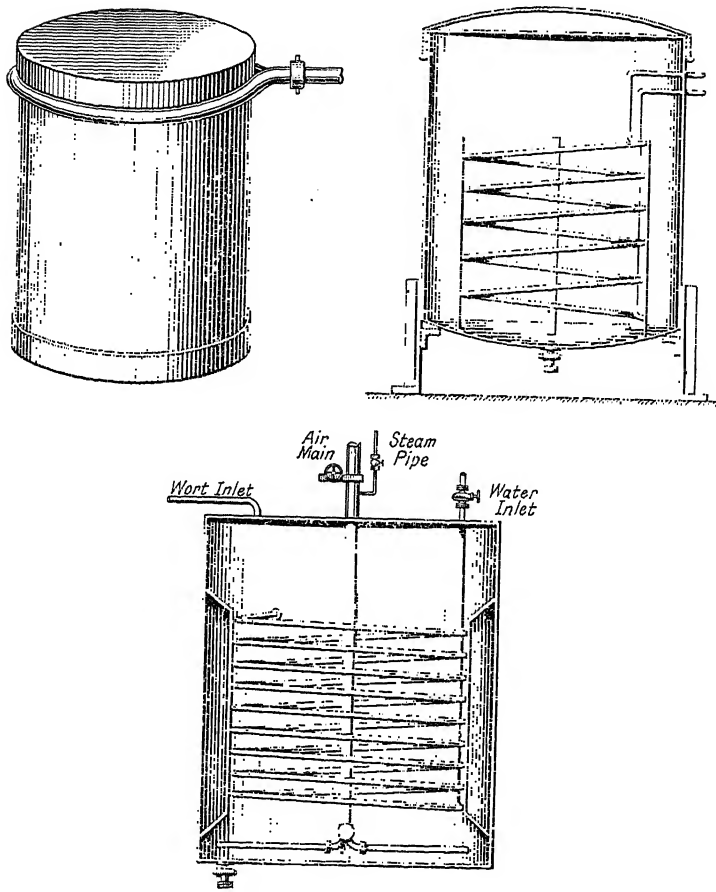


FIG. 4. Factory vessels required for seed-yeast culture.

The G6 culture is grown in an externally cooled copper vessel with a capacity of 29 litres.

The G7 culture is cultivated in a vessel of similar pattern, but with a capacity of 154 litres.

The G8 culture requires a vessel with a capacity of 837 litres and furnished with a portable cooling coil.

The G9 culture is aerated in a covered vessel of 4,815 litres capacity furnished with an air distributing system, cooling coils and steam service.

about 4 gallons of water at the same level as the culture, and when recording the temperature it is sufficient to observe the temperature of the water-bath, which is usually 1 degree below

that of the culture. The fermentation is complete in about eighteen hours, and at this point the culture is agitated with a sterile glass rod to disturb the sedimentary yeast for microscopic examination. The temperature of the culture is then reduced to 75° F. The attenuation acidity and alcohol content is determined in unfiltered samples of the wort and the results noted on the Seed-Yeast Culture Record.

SEED-YEAST CULTURE RECORD

Date..... Giant Colony..... Date Plated.....By .....

Generation	Started	Finished
1	Feb. 30th	Mar. 2nd
2	Mar. 2nd	,, 3rd
3	,, 3rd	,, 4th
4	,, 4th	,, 5th
5	Mar. 5th	Mar. 6th
Time .	3 p.m.	3 p.m.
Acid .	4·5	4·0
Alcohol .	—	4·45%
Gravity .	12·0 B	2·0 B.
Temp. F. .	73·5	80
6	Mar. 6th	Mar. 7th
Time .	3 p.m.	3 p.m.
Acid .	4·4	3·9
Alcohol .	—	4·63
Gravity .	12·2 B.	2·1 B.
Temp. F. .	75	80
7	Mar. 7th	Mar. 8th
Time .	3 p.m.	3 p.m.
Acid .	4·3	3·8
Alcohol .	—	4·5
Gravity .	11·9	2·0
Temp. F. .	75	80

Generation	Started	Finished
8	Mar. 8th	Mar. 9th
Time .	3 p.m.	3 a.m.
Acid .	4·5	3·9
Alcohol .	—	4.4%
Gravity .	12·4 B.	1·9 B
Temp. F.	75	80

Generation 9 Alcohol 3·0%

Started Mar. 9th. 3 a.m. Acid 4·4

Finished Mar. 9th. 3 p.m. Acid 3·7

Time	Temperature	Gravity.
3	77	9·0
4	77	7·6
5	78	7·4
6	78	7·0
7	79	6·6
8	80	6·0
9	81	5·2
10	82	4·2
11	83	3·0
12	83	2·3
1	84	1·9
2	83	1·8
3	82	1·8

G6 Culture. The G6 container has a capacity of 95 litres and the following dimensions: Diameter, 45 cm. (17·75 inches); depth, 60 cm. (24·5 inches), and ample capacity to accommodate the 48 litres of sterile wort and the 16 litres of fermented wort transferred from the G5 container. After the vessel has been washed, scoured with hot water and steam-sterilized for thirty minutes, it is drained of condensate with the cover in

position and immediately filled to the 48-litre mark with hot wort from the filter tank. The acid value of the wort should be between 4° and 5° and the gravity 12° Balling. The wort is chilled to (24° C.) 75° F. by spraying the sides of the vessel with cold water, and inoculated by adding the 16 litres of G5 culture after agitation and examination.

The temperature is permitted to rise to 80° F. in eight hours, remaining at that until the end of fermentation, when it is reduced to 75° F. The temperature is observed and noted on the record, and the acid, alcohol and gravity Balling determined in the fully fermented wort.

At this point the alcohol production, attenuation and acid consumption of the culture is compared with records of previous cultures and any marked variation noted. It is an established fact that if a number of cells are isolated from a culture derived from a single cell the cultures produced from these isolated cells will often show considerable differences, under identical conditions of fermentation, in regard to alcohol production, attenuation and fermenting power. These changes in the yeast occur without obvious cause and influence the quality and characteristics of commercial yeast produced from a single cell. This undesirable feature is avoided by selecting twenty colonies from the Petri dish culture when isolating the yeast, thus producing a multi-cell culture with compensating differences. But if any variation in the properties of the yeast should occur it is sufficiently developed in the G6 culture to be recognized.

G7 Culture. The G7 container has a capacity of 375 litres and the following dimensions : Diameter, 75 cm. (29·5 inches) ; depth, 85 cm. (33·4 inches), and allows the customary one-third of its capacity for freeboard. The vessel is discharged by means of a plug cock fitted on the bottom, and is mounted on an iron stand to command the G8 container, which, in turn, is superimposed on the G9 fermenter so that these cultures gravitate from one to another. In order to avoid pumping sterile wort the culture vessels may be placed near the wort cooler from the top of which the hot wort flows, or is syphoned, into the vessels as required.

The G7 container is filled to the 192-litre mark with hot

sterile wort at 12° Balling, and, after cooling to 75° F., is inoculated by following the same procedure as applied to the previous cultures. The temperature is allowed to rise to 80° F. towards the end of the fermentation and is then reduced to 75° F.

The same day that G7 culture is inoculated the amount of raw material mashed in the routine brew is increased by 617 kg. to supply the extra wort required for the cultivation of G8 and G9 generations next day.

G8 Culture. The G8 container has a capacity of 1,557 litres and the following dimensions: Diameter, 115 cm. (45·2 inches); depth, 150 cm. (59·2 inches). The vessel is mounted upon a suitable iron stand and the centre of the dished bottom is fitted with a 1-inch plug cock riveted to the metal and sweated to a smooth finish. A 1-inch copper tube fitted with ground-in sanitary unions delivers the culture to the G9 fermenter.

Temperature control and cooling is effected by means of a portable copper coil, consisting of eight turns of 1-inch copper tube clipped to four vertical copper rods which space the coils 4 inches between centres and 4 inches off the bottom of the container. The inlet and outlet of the cooling coil is hooked over the top edge of the vessel the contents of which are protected from exposure by a metal hood riveted to, and extending from, the edge of the cover. The coil is connected to the cooling service, and to the drain, by means of hoses and hose connections. The vessel is easily cleaned by removing the coil, and, after the usual scouring, it is replaced, rinsed with a jet of hot water and the covered vessel steam sterilized for thirty minutes, the condensate draining away through the discharge cock.

The 617 kg. of extra raw material included in the previous day's routine mash provides the 768 litres of 12° Balling wort required for the G8 culture and also the 3,092 litres of 9° Balling wort for the G9 generation.

The G8 container is filled to the 768 litre mark from the wort delivery tube when the wort flowing over the cooler indicates 12·5° Balling and cooled to 75° F. by passing chilled water through the coils. The acidity and gravity Balling is

determined and, after the addition of 250 c.c. of 25% ammonia solution, the wort is inoculated by the addition of the 256 litres of G7 culture. The temperature of the fermentation is permitted to rise one degree per hour until it reaches 84° F., at which it is maintained until the end of fermentation and then reduced to 75° F.

From the beginning of the seed-yeast culture until the end of the eighth generation the yeast is cultivated in strong worts that produce between 4% and 5% of alcohol. In the last two stages, G11 and G12 differential brews, the yeast is grown in extremely dilute worts in which the reproductive activity of the yeast is stimulated to the utmost and the fermentative power is suppressed to produce a non-alcoholic wash.

Yeast has a marked ability to adapt itself to its environment but its properties also change with the conditions, and in order to produce a consistent commercial yeast any alteration in the conditions of its growth must be carefully controlled. The change from alcoholic to non-alcoholic fermentation is effected by gradually varying the conditions in the next three generations and at the same time a tolerance to inorganic nitrogen is developed by increasing the amount added in each brew.

G9 Culture. The G9 fermenter has a capacity of 6,500 litres (1,435 gallons) and is constructed according to the following specifications. Diameter, 213 cm. (83·8 inches). Depth, 182 cm. (72 inches). The sides and the top and bottom are made of $\frac{1}{8}$-inch steel plate, butt joints are welded and ground to a smooth finish, lapped joints are riveted and either welded or sweated smooth to fill any cavities, however minute.

The top edge of the tank is reinforced with an external ring of $1\frac{1}{2}$-inch angle iron, to which the cover is attached, and to ensure rigidity this is clipped to strips of 2-inch tee iron laid across the top of the tank. A 24-inch ring of angle iron frames the manhole and this is protected by a suitable dust proof sheet-metal cover.

The cooling coil consists of seven turns of $1\frac{1}{2}$-inch copper tubing spaced 6 inches between centres and 6 inches from the sides and bottom of the tank. The coil is supported by five copper straps, 2 inches by $\frac{1}{4}$ inch, to which it is clipped, with

$\frac{1}{4}$-inch gun metal hooks. Both top and bottom of the straps are bent at an angle to bolt on the sides of the tank.

The air-distributing system consists of a $4\frac{1}{4}$-inch copper air main passing vertically through the centre of the cover to a 3-inch horizontal main at the bottom of the tank; this pipe is fitted with fourteen unions, seven on each side, to which the 1-inch distributing pipes are connected. These pipes are perforated with two rows of $\frac{3}{32}$-inch (2·381 mm.) holes on the bottom, the total area of the 1,024 jets equal the cross section area of the 3-inch main. The open ends of the distributing pipes are closed with a screw cap, kept free of yeast by means of an air jet drilled through the centre.

A 2-inch hole is drilled in the tank cover to admit the wort pipe from the pump or cooler. The bottom of the tank is fitted with a 2-inch flanged plug cock and ground sanitary union connecting a 2-inch copper tube to the main fermenter. A 1-inch steam pipe is connected to the vertical air main outside the tank and between it and the air regulating valve. The steam is used in sterilizing the air mains and tank and also for heating the chilled wort to a suitable temperature before its inoculation.

Before use the tank is thoroughly cleaned, air pipes are dismantled, scoured inside with a flexible wire tube brush and the plug cock on the bottom of the tank is given close attention. When all the fittings are assembled and in position the interior of the tank is closely inspected, then closed, steam-sterilized for thirty minutes and opened only when it is necessary to attend to the wort and its inoculation.

The tank is charged to the 3,072 litre mark immediately after the G8 container is filled, and it will be found that with the normal reduction in gravity of the wort towards the end of filtration the final gravity of the G9 wort will be slightly above 9° Balling and very little dilution is necessary, but as this wort must stand for more than twelve hours, until G8 has fermented, it is kept sterile either by heat or by chilling it beyond the range of bacterial activity. Sterility is certain if the temperature of the wort does not fall below 168° F. during storage, but it should be chilled to 77° F. in the shortest possible interval before inoculation. In cold climates satisfactory

results are obtained by storing the sterilized wort at reduced temperatures in a sterile container, and in this example the wort is chilled to 60° F. and stored at that temperature until it is required for the G9 culture.

The pressure of steam available determines the period occupied in heating the G9 wort before its inoculation, steaming usually commences thirty minutes before inoculation so that the temperature of the sterile wort reaches 77° F., coincident with the end of the G8 fermentation. As soon as this temperature is reached a sample of the wort is taken, microscopically examined and the acidity and gravity determined. One litre of ammonia solution is added and the wort is inoculated with the previously examined G8 culture.

The contents of the tank are aerated by injecting 200 cubic feet of air per minute for the first three hours and increasing the volume to 350 cubic feet per minute for the period remaining until the wort is attenuated to 1·5° Balling. Aeration is then reduced to a volume sufficient to keep the yeast in suspension until it is discharged from the tank.

The temperature is allowed to rise from 77° F., one degree per hour until 84° F. is reached, at which it remains until the end of fermentation. Chilled water is then admitted to the coils to reduce the temperature to 72° F. before the culture is discharged into the G10 fermentation. The gravity Balling and the temperature are recorded each hour and, after fermentation, the acid and alcohol concentration is determined in the unfiltered wort.

G10 Culture. The G10 fermentation is conducted in the main fermenter by a method developed to produce a gross yield of 33% yeast and a reduction in the alcohol concentration to between 1·5% and 1·8% and an increase in the amount of inorganic nitrogen consumed by the yeast. The process is therefore quite different to any of the preceding generations and to any that follow it. In order to produce the necessary extract the full amount (1,371 kg.) of material is mashed and soured, according to the method detailed in the next chapter. The filtration of the wort from the mash varies from the differential brew routine and is necessarily delayed until the fermentation of G9 culture is nearly finished. For instance,

the filtration of the wort for the routine differential brews commences at about 9 a.m. but filtration of the G10 wort commences at about 1.30 p.m. to be brought under the protecting influence of the G9 yeast at 3 p.m. This procedure dislocates the factory routine if the G10 brew is fermented at any times other than as the last brew of the week because the mash remains in the tun and for obvious reasons is sterilized immediately before its discharge into the filter tank after mid-day.

About an hour before it is desired to commence wort filtration the mash is sterilized at 168° F. and discharged into the filter tank. After circulating the wort through the grains until it runs quite bright, it is pumped over the cooler and flows into the fermenter at 72° F. until this vessel contains, in litres, the equivalent of four times the weight of the grain used in mashing (1,371 kg. × 4 = 5,484 litres). Filtration then ceases until the addition of G9 culture to the brew.

The acidity and gravity Balling of the wort in the fermenter are determined and, with the time and temperature, recorded on the ferment chart; 5·5 kg. of ammonium sulphate in solution is added, the blower speed increased (this machine is feebly aerating the G9 culture) and the brew aerated by blowing 505 cubic feet of air per minute through the wort.

The fermentation commences with the addition of G9 culture to the fermenter, and whilst this vessel is discharging full air is admitted to disturb any sedimentary yeast that may have settled during the feeble aeration period. As soon as the flow of G9 ceases the delivery tube is disconnected and the small amount of residual yeast and wort washed down the drain.

The wort escape taps on the filter tank are opened and set to complete the filtration four hours after the addition of G9 to the fermenter. The total volume of the wort equals twelve times the weight of raw material mashed (1,371 × 12 = 16,452 litres) and at this point another 5·5 kg. of ammonium sulphate is added to the brew. It is important that the period of secondary filtration occupies the full four hours, any reduction in this period not only reduces the yield of yeast and increases the alcohol concentration in the wort, but stimulates

fermentative activity with the result that the commercial yeast produced from such seed differs from the standard in the dough and sugar tests.

The aeration and the temperatures of a G10 brew are controlled according to the following table :—

Aeration.			Temperature.
Time.	Cu. Ft. Air per Minute.	Fraction of M^3 per Hour.	
When G9 culture is added .	505	0·625	One hour at 72° F.
When deg. Balling is 6·5 .	604	0·75	Two hours at 72·5° F.
When deg. Balling is 4·5 .	726	0·90	One hour at 75° F.
When gravity is constant .	505	0·625	Then rises 2·5° F.
One hour after . . .	Slight	—	per hour to 86° F.

The volume of air necessary for the complete oxidation of brewing sugars to CO_2 and water in a non-alcoholic fermentation is found to be not less than 1 cubic metre of air per hour per kilogram of raw material brewed, calculated for the full fermentation period, but this volume is far too great for the G10 fermentation in which a small but definite yield of alcohol is necessary and fractions only of a cubic metre of air are injected into the brew, as indicated in column 3 of the above

Ferment Chart

DATE..... BREW No..... GENERATION G10. MASHED by.....

MATERIALS		CHEMICALS.		MASHING AND SOURING.					FILTRATION.
	Kilos.		Kilos.	Time.	Mash Acidity.	Gravity Balling.	Temperature deg. Fahr.	Mash heated to	
Maize .	—	—	—	10.30 a.m.	—	—	144	—	Time started, 1 p.m
Wheat	—	Lime	—	1.30 p.m.	1·1	—	133·5	—	Time finished, 7 p.m
Barley	205	$Al_2(SO_4)_3$	—	6.30 p.m.	1·9	—	129·5	138	Hours, 6
Tapioca	728	$(NH_4)_2SO_4$	11	10·30 p.m.	Stirred	5 min.	—	—	Gravity, 17·5° B.
Rice .	—	NH_4OH	—	2.30 a.m.	Stirred	5 min.	—	—	Mashed, 4.30 p.m.
Molasses	—	H_2SO_4	—	6.0 a.m.	Stirred	5 min.	—	—	
Wheat Malt	—	$CaH_4(PO_4)_2$	—	11.0 a.m.	—	—	134	168	
Chev. Malt	274								
Malt Culms	164								
Total	1,371								

Fermentation Record

Time in Hours.	Temp. deg. Fahr.	Gravity in degrees Balling.	Acidity in Brewers degrees.	*p*H.	Formalin Number.	Cubic Feet of Air per Minute.	Litres of Stove Wort added	Litres of NH_3.	Kilograms of $(NH_4)_2SO_4$	Litres of H_2SO_4	Litres of $CaH_4(PO_4)_2$ Solution.
1 p.m.	72	15·0	5·9								
3 ,,	72	10·2	4·7	4·5	1·8	505	Start second filtration.		5·5		G9 culture added.
4 ,,	72	9·2	4·5	4·4	—	505	4,389				
5 ,,	72·5	8·1	4·0	4·2	1·1	505	3,290				
6 ,,	72·5	7·0	3·6	4·0	—	604	2,193				
7 ,,	72·5	5·9	3·2	4·4	1·0	604	1,096		5·5		Filtration complete
8 ,,	75	5·2	3·0	4·2	—	726					
9 ,,	77·5	4·5	2·9	4·0	0·8	726					
10 ,,	80	3·6	2·7	4·2	—	726					
11 ,,	82·5	2·9	2·4	4·4	0·5	726					
12 ,,	85	2·4	2·0	4·6	—	726					
1 a.m.	86	2·0	1·8	4·6	0·3	726					
2 ,,	86	2·0	1·8	4·8	—	505					
3 ,,	85	2·0	1·7	5·0	—	Feeble.	Separation commenced.				
Separated wort		1·5	1·7								

Extract and Yields

Strong wort	5,484 litres at 15·0 deg. Balling	= 822 Balling hectolitres
Store wort	10,968 litres at 2·0 deg. Balling	= 219 ,, ,,
Total	16,452 (plus G9 = 20,548 litres)	1,041

Total raw material	G9 and G10. 1988 kg.
Yield of yeast.	597 kg. = 30% yield.
Alcohol concentration	1·79 per cent. = 367 kg. = 18·4% yield.

Dough test	58 minutes.
Sugar test	820 c.c.
Protein	51·4 per cent.

table, the volume increasing empirically with rising temperatures until the end of fermentation.

The time, acidity, and gravity Balling are recorded on the G10 ferment chart at intervals of one hour from the start to the finish of the fermentation. *p*H is determined every hour after the second, and the formalin number every alternate hour.

A departure from the temperatures indicated is unusual, but the fermentation should occupy the full period shown on the specimen ferment chart, and if attenuation appears to be too rapid at any time, the activity of the yeast may be retarded by a slight reduction in the temperature. The vigorous aeration of a low temperature fermentation generally causes voluminous froth, and the G10 brew is no exception to this rule. The installation of a fat-melting vessel is essential, and this may be suspended in the tank in such a position that as

the froth rises some of it flows into the vessel and displaces sufficient fat to disperse the froth for the time being.

One hour after the fermentation is complete, the brew is separated, the yeast cream chilled and filter pressed according to the methods described in the differential fermentation. The G10 yeast is packed in containers that are plainly marked with the generation number or some other distinguishing device in order to avoid its accidental use for other purposes. It is stored in the icehouse at temperatures below 40° F., and may be kept for a month and safely used for producing G11 yeast without any trace of degeneration.

A sample of the filter-pressed yeast is carefully examined under the microscope over the whole range of the cover glass, and any feature out of the ordinary should be noted. Infection of any kind immediately condemns the yeast for seed purposes. The dough test should be commenced as early as possible and the dough generally takes sixty minutes to reach the bar; any faster time than fifty-five minutes is exceptional and is not desired, as the differential fermentations following will further reduce the dough time to between forty and fifty minutes in the commercial yeast. The sugar test (Kusserows) will usually displace 800 c.c. of water in two hours. The nitrogen is determined by the Kjeldahl method, and protein (nitrogen × 6·25) averages 52·0%.

The alcohol is determined in the separated wort by distilling 100 c.c. of the neutralized wort into a 100 c.c. volumetric flask, and after weighing the distillate in a 50 c.c. specific gravity bottle the specific gravity is calculated and the percentage of alcohol obtained by reference to the alcohol tables. The wash from the separators is of little value and is generally discharged down the drain, although it may be fortified with spirits for the production of vinegar, or distilled to produce industrial alcohol.

CHAPTER III

MASHING; AND THE FILTRATION AND DIVISION OF WORT

In the production of compressed yeast there are two starting points: the pure culture flask supplying the yeast and the grist mill preparing the grain to nourish the yeast. In both sections the work proceeds along separate lines to a point where the products of both meet. The isolation and cultivation of a suitable seed yeast are as essential in yeast production as the careful selection of seed wheat is in agriculture, and for the same reason. In that industry, the best soil is prepared to supply plant foods in solution with the addition of nitrogen, potash and phosphates to correct natural deficiencies; and in yeast propagation the substrate is prepared and the foods in solution are supplemented with nitrogen, potash and phosphates when a deficiency occurs.

Mashing for the Differential Brew

Mashing is the process of extracting the valuable constituents from the raw and malted grain by mixing them with water at suitable temperatures and relative quantities, and in converting the dissolved substances into products suitable for the nourishment of yeast. Chemically it proceeds by the inversion of gelatinized starch into dextrin, malto-dextrin and maltose and the modification of the native proteins into soluble and diffusable forms. These changes are effected by the activity of enzymes, chiefly diastase and peptase, and commence when the grain is mixed with water at definite temperatures. The relative amounts of dextrin, malto-dextrin, maltose and assimilable proteins finally present in the wort depends upon the dilution, temperatures employed, hydrogen-ion concentration and other conditions, such as the time factor, during the mashing process.

Although the extract, or amount of solid matter extracted in solution from the mash, depends primarily upon the amount of carbohydrates contained in the grain, extraction is also influenced by the percentage composition of the mash. Sufficient malt is included to secure the maximum conversion of starch to brewing sugars, but the grain residues, husks and culms must be in such a condition that in the mass they form an efficient filter to retain the finest particles of solid matter and yet permit the free flow of wort from the filter tank.

Filter efficiency is largely determined by the nature of the raw materials and the degree of fineness to which it is crushed before mashing. Several varieties of grain are usually employed; tapioca, wheat, or maize for its high percentage of starch; barley for its extract and the filter value of its husks, and culms are essential as a source of nitrogen and as filter material. It is possible to use one kind of material only, malted and raw wheat for instance, and with culms to secure a complete extraction by crushing the grain in such a manner that the starch body is exposed to gelatinization and conversion without shattering the husk, which then assists the culms in forming a porous filter.

Relative Value of Raw Materials. Brewing with one kind of grain is rarely practised however, and then only when the relative cost of the various raw materials renders this course necessary in the interests of efficient and economical brewing, but in considering this feature the cost of crushing, which varies widely with different grain, must be added to the prime cost and transport charges. The table on p. 67, calculated from the average extract and bushel weight, shows the relative value of the various materials used in brewing for yeast production.

Malt culms are not included in the table because extract alone does not determine their value, their importance as filter material must also be considered, and in estimating the value of samples of this material special tests must be applied and compared with similar tests of a standard sample. The extract value of malt is shown, but for comparative purposes only, for the reason that in cost estimation the extract in malt is secondary to its diastatic activity.

Relative Value of Raw Materials

Grain.	Extract.	Lbs. per Bushel.	Vertical Columns show the Relative Values of Raw Material, according to Extract, in Pence, per Bushel.																		
Barley . .	50	50	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
Barley . .	55	50	26	28	30	33	35	37	39	41	44	46	48	50	52	55	57	59	61	63	66
Barley . .	57·5	50	27	29	32	34	36	39	41	43	46	48	50	53	55	57	59	62	64	66	69
Barley . .	60	50	28	31	33	36	38	40	43	45	48	50	52	55	57	60	62	64	67	69	72
Maize . .	65	56	34	37	40	43	46	49	52	55	58	61	64	66	69	72	75	78	81	84	87
Rye . . .	65	60	37	40	43	46	49	53	56	59	62	65	68	71	74	77	81	84	87	90	93
Wheat . .	70	60	40	43	47	50	53	57	60	63	67	70	73	77	80	84	87	90	94	97	100
Oats. . .	40	40	15	16	17	19	20	21	23	24	25	26	28	29	30	32	33	34	35	37	38
Tapioca . .	85	112 (cwt.)	91	99	106	114	121	129	137	144	152	159	167	175	182	190	198	205	213	220	228
Cape Malt. .	68	40	25	27	29	31	33	35	37	39	41	43	46	48	50	52	54	56	58	60	62
Chev. malt .	70	40	26	29	31	33	35	38	40	42	44	47	49	51	53	56	58	60	62	64	67
Wheat malt .	80	40	30	33	35	38	40	43	46	48	51	53	56	58	61	64	66	69	71	74	76

Tapioca roots, with 85% extract, are probably the cheapest raw material. The dry, crescent-shaped tubers vary somewhat in dimensions but average about 6 inches in length and 1 inch in diameter. The roots receive a preliminary crushing in a coke mill, or similar apparatus, to reduce them to a suitable size to pass through the feed rolls of an ordinary roller mill for final grinding, but if tapioca is too finely divided it retards filtration in a marked degree.

Wheat is finding an extended use in yeast brewing, and produces the best results when the grain is rolled into flakes. Owing to the presence of gluten this cereal requires slightly different treatment in the starch cooker ; the flakes are steeped in water at 125° F. with 1% of malt, the temperature is then raised to 140° F. by the injection of live steam and kept at that degree for 60 minutes. The mixture is then boiled and as soon as this temperature is reached, the mucilage becomes quite fluid and may be immediately added to the malt in the mash tun.

Barley has been used as a brewing material from time immemorial, because the husk protecting the acrospire facilitates steeping, aeration, and kilning during malting operations, and serves as valuable filter material in brewing processes. In addition the raw barley slowly gelatinizes at mash-tun temperatures, although improved results are obtained when the barley mash is preheated to 160° F. before its addition to the malt in the mash tun. Barley is crushed according to the type and moisture content ; Cape barley flakes readily without injury to the husk, but careful milling does not prevent shattering the thin and brittle husk of Chevallier barley.

Maize intended for gelatinization in an open vat should be ground to a fine meal, a condition best obtained by the use of a disintegrator or emery grinder. Crushing this grain in a roller mill is both slow and costly. The meal is gelatinized in the starch cooker with 1% of its weight of malt, from 3½ to 4 times its weight of water at 125° F., and sufficient sulphuric acid to adjust *p*H at *p*H 5. After boiling the mucilage should be quite fluid before its addition to the malt in the mash tun, otherwise an appreciable amount may be found choking the grains in the filter tank next day.

Maize is treated most economically by gelatinization in a pressure vessel of the Henze or Hollefreund type, to which the grain is added without preliminary crushing and, after soaking in water overnight, boiled for a short period at atmospheric pressure. The manhole is closed and the pressure increased to between 40 and 50 lb. per square inch. The pressure is then reduced to that necessary to blow the mucilage into the mash tun. When it leaves the pressure vessel the mucilage passes through a comminuting valve which effectively reduces the horny residues of the corn to a very fine state of division.

Oats are not used for their extract value in brewing, but if supplies can be obtained cheaply this grain is a valuable aid to filtration, and may be used to advantage with wheat or tapioca. Oats are flaked easily in a roller mill and readily saccharify at mash-tun temperatures.

In the composition of the example brew shown below, tapioca forms the chief ingredient (53% of the total material), but either maize or wheat may be used with, or in place of, tapioca.

Malted wheat .	10%	302·4 lb.	= 137·14 kilograms
Malted barley .	10%	302·4 ,,	= 137·14 ,,
Raw barley. .	15%	453·6 ,,	= 205·71 ,,
Tapioca roots .	53%	1,602·7 ,,	= 726·84 ,,
Malt culms . .	12%	362·9 ,,	= 164·5 ,,
	100%	3,024	1,371·33

The portion of the brewing material that gelatinizes at temperatures above 144° F. (tapioca, maize or wheat) is treated separately in a vessel, termed the starch cooker, that commands the mash tun so that after gelatinization the hot mucilage flows by gravity into the malt mash below. The gelatinization of coarsely crushed grain is not instantaneous at boiling temperatures, and it is necessary to treat the raw grain first and allow it to stand at high temperature whilst the malt mash is in process.

In order to secure the maximum conversion of the starch to sugar the raw grain is gelatinized and liquefied in the starch

cooker before its addition to the malt. The mucilage should flow as a smooth liquid without any lumpiness; a viscid mucilage cannot readily saccharify and much of the difficulty experienced in wort filtration is due to the incomplete treatment of raw starches. In a pressure vessel the fluidity of the mucilage is assured, but in an open starch cooker partial liquefaction is effected by enzyme activity at the optimum *p*H and suitable temperatures. The starch cooker is charged with a volume of water equivalent to 3·5 times the weight of the material to be processed; 1,602 lb. of tapioca plus 16 lb. of malt = 1,618 lb. of material = 566 gallons of cold water. This is heated to 125° F. by the injection of live steam, or an equivalent volume of hot water may be used from the hot water supply. Wheat malt in an amount corresponding to 1% of the weight of raw grain under treatment is added to the water in the cooker, the vat covers are then closed, and with constant agitation the crushed tapioca is added in a continuous stream from the bin, or if from sacks, as uniformly as circumstances permit. The cold tapioca reduces the temperature of the charge and to prevent the formation of a starch sludge on the bottom of the vat the temperature of the mixture is kept constant by the cautious injection of live steam.

When the vessel is charged with the full amount of tapioca, *p*H is determined in a filtered sample of the liquid and the hydrogen-ion concentration adjusted at *p*H 5 by the addition of sulphuric acid. Steam is then admitted and the temperature of the mixture increased to 139° F., at which it remains for from one to three hours with occasional stirring. During this interval enzyme activity is demonstrated by a considerable increase in the amount and density of the liquid portion of the mixture. The mash is then agitated and the temperature raised to the boiling point, at which it stands until mashing begins. Active ebullition of starch mucilage commences at about 200° F., accompanied by considerable frothing; between this point and 212° F. the vat requires close attention to avoid overflowing.

A fluid mucilage may in some cases be obtained by quickly increasing the heat from 125° F. through the whole range of gelatinizing temperatures to boiling point, but owing to the

diverse nature and condition of the materials employed in yeast brewing, this method often fails when a slow filtration is most disastrous. This is largely due to the different gelatinizing temperatures that often occur in the one kind of grain ; for instance, a dry friable grain has a porous structure, readily absorbs moisture, and yields a fluid mucilage with rapid heating, but moisture penetration is much slower in a hard flinty grain of the same kind, and a viscous mucilage is inevitable when this is gelatinized without proper treatment. It is found that with grain in this condition some of the starch resists boiling temperatures before the granules are ruptured by partial gelatinization.

The Mash Tun. Whilst the raw grain under treatment is in process of gelatinization a malt mash is prepared in the mash tun to complete the mashing process.

The mash tun is charged with a volume of water equal to three and a half times the weight of the grain to be treated ; 588 lb. of malt plus 453 lb. of raw barley = 1,041 lb. of grain = 364 gallons of water. The agitators are geared to rotate at half speed to avoid shattering the husks and the grain is delivered to the mash tun in a continuous stream until the bin is empty. The emptiness of the bin is confirmed by inspection, as crushed husky grain sometimes forms a scaffold retaining some of the grist in a corner of the bin.

When the addition of the grain is complete, the temperature of the mash is adjusted at 112° F. and gently stirred for a peptonizing period of sixty minutes. During this interval a proportion of the " native " proteins is dissolved and hydrolysed to peptones and amides. Other complex reactions also occur that materially influence the extract as demonstrated by the difference between two test brews, one of which is saccharified without peptonization and the other peptonized and saccharified. The latter will invariably produce a better extract without any apparent difference in filter efficiency.

After the mash is peptonized, chilled water is admitted to the cooling coils, and with full agitation the contents of the starch cooker are added to the mash tun as rapidly as possible, but with temperatures carefully controlled to preserve diastatic activity.

Diastase is not destroyed by acting upon starch, and in comparatively pure solutions it will hydrolyse many times its own weight of starch. When the reaction is complete the amount of diastase will be found the same, but mash-tun worts cannot be considered pure solutions in this respect, for there are substances introduced with the grain—iron for instance—that progressively retard, or inhibit, diastatic activity, with the result that a better conversion is obtained when the mucilage is added quickly than when the addition is delayed. This requires careful temperature control, however, for if the hot starch is delivered in too great a volume there may be sufficient local heating at the point of contact with the mash to destroy the diastase there. For this reason the hot starch is at first slowly sprayed on the surface of the mash, and as the volume of the mash increases the flow of mucilage is correspondingly increased.

With an adequate supply of cooling water flowing through the coils, about three-fourths of the gelatinized starch is delivered before the temperature of the mash reaches 135° F. The flow from the starch cooker is then adjusted so that when all the mucilage is added the mash temperature indicates 139° F.

In winter with cold service water the mash may be completed in twenty minutes, but in summer when the service water temperatures are so much higher, mashing may be prolonged until it is completed with exhausted diastase. In localities where cold river or well water is available, or in plants equipped with an ice machine of sufficient capacity to provide chilled water for cooling the mash in hot weather, none of the difficulty is experienced in mashing that is found where the cooling service is inadequate. In hot climates various expedients are adopted to overcome cooling difficulties. Boiling the raw grain a day previous to mashing and allowing it to cool overnight is one successful method. The mucilage rarely falls below 160° F. after sixteen hours' standing, and thus allows the mash to be prepared without using any cooling medium.

Cooling troubles may also be avoided by using materials that gelatinize at low temperature, and where supplies of

barley are available the mash may be composed of malted and unmalted barley ; the raw grain is gelatinized in the starch cooker at 160° F. and then added to the malt in the mash tun with a very small consumption of cooling water, or, if the raw grain is dry and friable, the gelatinization of the raw barley and mashing may be combined in the mash tun. Cooling is thus eliminated and the starch cooker dispensed with, but the former method is most efficient and produces the better extract.

For the observation of correct mash temperatures a bucket type thermometer holder is used, consisting of a copper bowl, about 3 inches in diameter and 2 inches deep, in which the thermometer bulb is immersed. The bowl is attached to a tubular guard containing the stem of a sensitive thermometer graduated from 80° to 180° F. A chain attached to the guard enables the bowl to dip portions of the mash when it is low in the vat, and thus the correct temperatures are observed. Angle thermometers, with the graduations outside and the bulb projecting through the staves into the mash, have not proved altogether successful, owing to the grains and culms lodging around the bulb and the temperature then indicated may vary widely from the true temperature of the mash.

Whatever facilities exist for the control of mashing operations, the temperature is not permitted to exceed 139° F. at any period when adding the gelatinized starch, and while the empty starch cooker is still hot it is sprayed with a jet of hot water to dislodge the residual starch adhering to the staves and agitator. These washings flow into the mash tun ; a more exhaustive cleaning then follows and the waste runs to the drain. If the vessel should cool sufficient for the mucilage to harden cleaning becomes laborious.

After the delivery of the mucilage to the mash tun, *p*H is determined in a sample of the wort and adjusted at *p*H 5 with lime or sulphuric acid as required, but any adjustment is rarely necessary when the *p*H of the starch mucilage has been corrected. The temperature of the agitated mash is then increased to 144° F. in order to gelatinize the malt starches. At this point the culms are added to the mash, either by tipping them direct from the sacks into the mash or, which

is cleaner, through a shute from one of the grain bins. The culms quickly absorb the excess moisture, and the last portions ride on top of the mash and gravitate towards the centre spindle in a compact mass that will not mix; this cake is broken with a rake or a jet of water and once moistened is rapidly dispersed.

With the addition of the culms a change is observed in the appearance of the wort, which, previously opalescent and turbid, is now comparatively bright and more highly coloured, a change that is partly due to the finely divided particles of the grain previously suspended in the wort adhering to the culms as they absorb moisture. If culms had not this property it would be difficult to filter bright wort from any grain other than barley. The addition of the culms completes the mash and the temperature is reduced to 139° F., at which the mash stands for the ninety minutes' saccharifying period. At temperatures below 140° F., where the diastatic activity is not weakened, more maltose and less dextrin is formed and the diastase continues to act on the dextrins, changing them to maltose. Thus the longer the mash is held at the optimum conversion temperature the greater will be the amount of maltose formed in proportion to the amount of dextrin. At the end of the saccharifying period the filter efficiency of the mash is determined and the starch conversion checked with iodine.

The filter value of the mash is quickly determined by means of a graduated sheet metal cone, of about 2 litres capacity, enclosing a close-fitting perforated sheet metal liner, both fitted with rigid handles. The apparatus is dipped into the agitated mash, withdrawn full and placed in a level stand, the perforated liner is then suspended so that the filtrate is retained, and its volume measured, in the graduated cone. The operation takes but a few moments and quickly indicates the condition of the mash. If conversion is complete and milling all that is desired, the wort will immediately drain away and leave the grains clean and crisp. Fine grinding is indicated by the debris filling the perforations in the filter and retarding the wort flow to a point where it may even cease, but the grains on the surface still appear clear and crisp. A retarded wort

flow is also caused by incomplete conversion, but in this case the grains appear soft and glutinous, and may even be covered with a film of gelatinous matter.

In any case the starch iodide test is applied and quickly reveals the presence of starch or the higher dextrins. A little of the unfiltered mash is taken in a test-tube, and, after cooling, 10 drops of the iodine solution are added, the tube shaken and the colour noted. If conversion is complete, the colour remains practically unaltered, but the familiar blue-black colour will indicate the presence of starch and a purple colour that of dextrins capable of further conversion.

The conversion of starch to sugar at this stage is a difficult matter and depends upon the kind of starch and its condition. If the presence of unaltered starch is due to the use of malt with feeble diastatic power the obvious remedy is to add sufficient high diastatic malt to complete the conversion, but with the exception of cereals, which gelatinize at mash-tun temperatures, such as barley or oats, all other unmalted grain will cause trouble if it is merely mashed without previous gelatinization. This may occur in error through faulty milling arrangements ; a few pounds of starch, such as may remain in an elevator boot or sometimes be found lining a screw conveyor, may not alone interfere with filtration in a marked degree, but these accumulations may be dislodged and swept together into the mash to give an iodine reaction that cannot otherwise be accounted for.

When a starchy condition of the mash is experienced without any apparent reason it may be advisable to investigate the raw material, especially tapioca roots, for sulphur dioxide or sulphites. At certain seasons of the year tapioca becomes infested with an objectionable stinging weevil peculiar to the tropics, and when these insects appear the authorities insist upon fumigation and occasionally use SO_2, with disastrous results to diastase and brewing operations. Carbon bisulphide is now more frequently used and proves quite harmless in the mash tun.

Souring the Mash. The production of lactic acid in the mash through the activity of specific organisms is not the only object of the souring process. Yeast only assimilates

proteins that diffuse through the cell wall and unless the native proteins in the mash are hydrolysed by suitable treatment to a diffusible form of yeast nourishment, they not only represent so much waste material, but coagulate and remain as particles of foreign matter with the yeast when it is separated from the wash. The peptic enzymes are assisted by the lactic acid formed in the mash, and in consequence more of the proteins are dissolved in a sour mash than in a sweet one, and more of those already dissolved are reduced to assimilable modifications.

In souring the mash, an operation that immediately follows the saccharifying period, the method recommended by Professor Delbruck and Dr. Lange is adopted. The temperature is reduced for a few hours to promote the rapid multiplication of suitable bacteria and then raised to a temperature that favours protein hydrolysis in contact with lactic acid. The optimum temperature for this transformation is about 104° F., but in the souring process this temperature is objectionable in that it favours the growth of undesirable micro-organisms and wild lactic acid bacteria; therefore higher temperatures must be employed. This feature has received close attention, and investigations showed that in contact with 2 degrees of lactic acid the percentage of diffusible protein formed in the mash between 135° and 140° F. is equal to that formed at 104° F. by the earlier methods of souring, and that the total amount formed is in proportion to the time that the mash remains between these limiting temperatures.

The souring process is an important feature, and a record is kept showing the progress of the work. The mash is agitated, the gravity Balling and acidity determined in a sample of the filtered wort, and the results noted on the ferment chart with the time and mash temperatures. The total acidity at this stage, immediately after the saccharifying period, will vary between 0·5 and 0·9 degree acidity.

The mash temperature is then reduced to 132° F. and to accelerate acid production, the lactic acid bacteria naturally occurring on the grain and culms are supplemented by the addition of a sour 'seed' consisting of 2 gallons of the previous mash, removed before sterilization and stored in the icehouse

until required. Examined microscopically, this seed is found to be almost a pure culture of *B. Acidi Lactici* by temperature selection. Routine pure cultures of this organism were prepared for mash inoculation until investigation proved that, at the temperatures employed in mashing, the lower types were destroyed, and that only the previously isolated and cultivated type survived.

Stimulated by the temperature and sour seed, the development of lactic acid is so rapid that after five hours' preliminary souring the mash is stirred, the acidity is determined, noted on the ferment chart and the temperature of the mash increased to 138° or 139° F., according to atmospheric conditions, for the secondary souring period. The mash-tun covers are closed, the steam valve checked and the mash stands, with occasional stirring, for from twelve to fifteen hours. At the end of this interval it is found that the acidity has increased to between 7 and 9 degrees acid.

While the mash is standing, usually overnight, the surface cools more rapidly than the bulk, and if it is allowed to remain undisturbed for the full period the temperature of the surface liquor is reduced to about 100° F., mainly by water vapour condensing on the covers and falling back upon the mash, which may then become coated with mould growths and tainted with their products. This unpleasant development is avoided by periodically stirring the mash during the souring period to equalize the temperature. Four minutes' agitation every four hours is found sufficient for the purpose.

If it is not practicable to arrange attendance to stir the mash at the above-mentioned intervals, condensation and reduction in the temperature of the surface liquor may be minimized by temporarily insulating the vat covers with a suitable heat-retaining material. As an alternative the mash may be composed of raw material with a protein content so low that protein hydrolysis becomes relatively unimportant, and the souring process is then directed to maximum production of lactic acid.

In this case the souring may be simplified by eliminating the primary souring period ; the mash temperature is adjusted at between 137° and 140° F. immediately after the saccharifying

period, the sour seed is stirred in and the mash stands for twelve or more hours without further attention. The vat covers are insulated to retain the heat, otherwise radiation and evaporation may reduce the temperature of the mash to below the minimum (130° F.) when souring is finished. If this should occur the initial souring temperature is raised in succeeding mashes, provided that the souring limit (140° F.) is not exceeded. Investigations proved that at 140° F. the activity of *B. Acidi Lactici* is entirely suspended, and that if this temperature is maintained for twelve hours the organism is destroyed.

One disadvantage of this method is its uncertainty ; sufficient acid may or may not be developed. If a deficiency does occur, it may be due to the maintenance of the initial souring temperature over too long a period, or a leaking steam valve prevents the normal fall in the souring temperature. When insufficient acid is developed in the mash, nothing can be done to rectify the matter but to add sufficient sulphuric acid to correct the deficiency and proceed with the work as usual.

Sterilizing the Mash. At the end of the secondary souring period the mash is agitated for five minutes and 2 gallons of the liquid portion are rough filtered into a suitable container and removed to the icehouse, where it remains until required as sour seed for the succeeding mash. After noting the temperature of the mash, the gravity Balling is determined in a 500 c.c. sample of the filtered and chilled wort and recorded with the result of the acid titration on the ferment chart.

The mash is sterilized by heating with live steam to 168° F., and, after standing at that temperature for fifteen minutes to destroy the lactic acid bacteria, it is discharged into the filter tank where the bright wort is filtered from the grain residues and conveyed to vessels in which it is stored and fermented.

Whilst the agitated mash is in process of sterilization the filter tank is prepared for its reception. The tank and fittings are flushed with water, filter plates are laid with the overlapping joints in position to retain the grains, wort escape taps are shut and the manhole closed and sealed.

The mash is at first slowly discharged into the filter tank until the filtrate completely covers the false bottom, where it is not covered with grains; this procedure is necessary in order to avoid the formation of an air lock under the filter plates. The mash is then discharged from the mash tun as rapidly as possible. If the tun is correctly set with an inch fall to the discharge cock and with the agitator blades just clearing the vat bottom it will completely discharge the mash and only the grains adhering to the sides of the vat will remain; this residue is dislodged with a jet from a hose and discharged into the filter tank with the minimum amount of water. The mash tun is then cleaned and the washings diverted to the sewer.

Wort Filtration and Division. The first filtrate that issues from the wort escape taps is turbid and carries with it the fine particles of debris that pass through the slots in the filter plates and lodge as a sludge, called 'underdough,' on the bottom of the tank. To clarify the wort and dislodge some of this underdough a by-pass and delivery tube are fitted on the wort delivery pipe line, so that the turbid filtrate may be returned to the surface of the grains in the filter tank, and thus circulated until the wort runs bright and clear. The wort escape taps are opened widely for a few seconds to allow a full flow of wort, and the taps are then sharply closed so that the recoil of the wort disturbs the sludge in the tank; this is repeated several times and the taps are then gradually opened until the proper flow of wort is set.

The correct setting of the wort escape taps is indicated by a filter gauge consisting of a vertical glass-tube, 1 inch in diameter, attached to the filter tank and with its lower end inserted in an angle valve; this is connected to a horizontal tube of equal diameter entering the tank under the false bottom. The upper end of the glass tube is open and a steam connection is made between the angle valve and the filter tank to allow steam to be blown through the tubes for cleaning purposes.

The advantage of this gauge lies in the fact that the flow of wort can be perfectly regulated. A suction under the false bottom, created by an excessive flow of wort from the taps, is indicated by the wort in the gauge dropping to a lower level

than that in the filter tank or disappearing entirely ; in this case the suction would be apt to choke the slots in the filter plates with grain and decisively retard filtration. It therefore indicates the perfect setting of the taps necessary for efficient filtration.

The wort is circulated through the grains for about thirty minutes before it runs bright. In the meantime the wort cooler, wort pipe lines and wort store tank are closely inspected, microscope slides are prepared from smears taken with a platinum needle from crevices and corners that may escape cleaning and sterilization, and if live organisms are detected the infected unit is cleaned and steam sterilized with formalin until it is bacteriologically clean.

Absolute sterility is essential in everything with which the wort comes in contact, small colonies of wild yeast may be found in the corners of pipe unions. Plug cocks are a fertile source of infection, especially on the washer and nut that secures the plug in position. The plug itself may be hollow, and, if so, is always viewed with suspicion unless cleaned in a hot soda bath, followed by a steam formalin spray. Wort pipes are made in straight sections, joined with sanitary unions to facilitate cleaning, inspection and assembling. Bends and elbows that cannot be inspected internally are made short enough to fit in a soda bath and sterilizing chamber.

The wort cooler receives close attention, particularly if it is the Baudelot type constructed of horizontal tubes sweated into end castings, termed headers, and supported by metal standards resting in the catch tray. The headers may be left rough by the founders and should be examined for the presence of minute blow-holes ; when these are found they should be filled with solder and left with a smooth finish. The standards resting on the catch tray are then sweated to the tray with a smooth wiped joint ; and any fabric jointing on the end plugs or fabric insertion in joints are replaced with sheet lead. The examination of the interior and fittings of the wort store tank then follows, paying the same close attention to top and bottom joints of the tank, the float and chain, discharge cock and wort delivery pipe.

The necessity for such meticulous care in an effort to

cultivate the yeast in sterile wort is apparent when it is realized that the conditions under which the yeast grows in a turbulently aerated differential fermentation is not only as favourable for the growth of many wild yeasts and moulds as it is for the yeast, but also provides the optimum conditions and substrate for the rapid multiplication of all strongly aerobic micro-organisms, and if vigilance is relaxed in the control of cleaning operations it is possible to produce a commercial article that contains more infection than yeast.

After inspecting the wort store tank, pipe lines, and wort cooler, the valves on the latter unit are opened to admit service water to the top section of pipes, and ice water or brine to the bottom section. Descending to the filter tank, fine wire sieves are placed in position over the wort pump suction box to retain particles of grain that may be discharged with the wort from the escape taps. The clear wort is then diverted to the wort cooler by closing the circulating by-pass at the top of the filter tank.

From the wort cooler the wort flows into the wort store tank at a temperature not exceeding 60° F. At or below this, or above 160° F., the growth of any infection, which may be caused by air currents carrying individual cells in contact with exposed wort, is retarded and thus more easily suppressed by the vigorous fermentation that follows.

After thirty minutes' filtration the grains begin to show through the liquor in the filter tank, followed by a slightly diminished flow from the wort taps. During this interval the service water flowing through the top portion of the cooler becomes heated by exchanging temperatures with the wort and partly fills the hot water tank at a temperature between 148° and 152° F., this is further heated to 180° F. with live steam and sprayed, or " sparged," on the surface of the grains to wash out the residual extract until the grains are exhausted. When all the wort disappears from the surface the sparge valve is opened and the full area of the grains is covered with a fine spray of hot water. The sparge is regulated to avoid the appearance of surface water above the grains, but the supply is sufficient to keep them in suspension to avoid a heavy subsidence of grains on to the filter plates. This pro-

cedure is continued until the original strong wort is diluted with the full volume of weak filtrate required and the last traces of extract are washed out of the grains.

In some cases the filtration is not always as efficient as desired, and before the original strong wort is discharged the grains drain imperfectly with a sadly diminished flow from the wort taps. This condition may result from any one or more of the causes described in the mashing process and should be indicated by the iodine or filtration tests after mashing. When this difficulty develops the method of sparging is adapted to meet the circumstances and a successful filtration may be secured by alternate flooding and draining. In this case the sparge valve is opened to its full capacity until the grains are covered to a depth of about 6 inches, it is then shut off until the grains are again exposed by filtration and the procedure is repeated until the grains are exhausted. This method may become such a time-consuming operation, however, that dislocation of the factory routine is threatened, and in this case an expedient termed "mashing" is applied.

The wort escape taps are shut, the grains are sparged and the underlet opened to lift the grains off the filter plates with hot water until the tank is filled to within 4 or 5 inches of the top. Long-handled wooden, or iron, rakes are then used to agitate the contents of the tank until a perfect suspension of the grains in the liquor is secured. The wort taps are opened and the turbid filtrate circulated until, when it runs bright, it is diverted to the store tank or fermenter as required.

In some cases it may be necessary to mash the grains when the filtration is rapid. It is sometimes found that channels are formed in the grains through which the sparge water passes directly to the filter plates, leaving a considerable amount of extract in other places where the grains have compacted and resist the flow of sparge water. This condition is indicated by a rapid fall in the density of the wort and the grains are mashed to ensure that at least 95% of the total extract from the mash will be delivered to the store tank in the volume of wort specified.

The total volume of wort required and its division between the wort store tank and the fermenter is governed by the fermenting period most suited to the factory equipment, and this may be designed to complete the fermentation in any period between ten and sixteen hours. The thirteen-hour fermentation is most economical in plant and labour charges and produces a nett yield of 90% yeast with a 14 to 1 gr. dilution. In the example under review the extract is diluted with a volume of water equivalent to fourteen times the weight of the raw material used in the mash : 1,371 kg. of raw material × 14 = 19,194 litres of wort.

If the period of fermentation is reduced, the dilution is greater, more seed yeast is necessary and the hourly volumes of air must be increased in proportion to the increased amounts of sugar to be oxidized. Conversely, a lengthened fermentation period allows a reduction in the dilution, amount of seed yeast and air volumes, but this involves divided control of the fermentation and the overlapping of one day's work with another.

For reasons connected with efficient aeration it is necessary to commence the fermentation with the greatest practicable volume of wort in the fermenter, but this must not contain more extract than the seed yeast can ferment in the hour before the differential flow of strong wort commences. It is extremely dilute and contains not more than 5% of the total extract. The store wort is therefore comparatively strong and contains the balance (95%) of the extract. This division of extract is obtained by dividing the wort into the volumes shown below :—

1,371 kg. × 14 = 19,194 litres of wort.

Store wort = 6/14 of 19,194 litres = 8,226 litres of the strong wort and first filtrate conveyed to the wort store tank at 60° F. Starting wort = 8/14 of 19,194 litres = 10,968 litres of the weak filtrates conveyed to the fermenter in which the fermentation is conducted.

The wort store tank is a totally enclosed sheet metal vessel 182·8 cm. in depth (72 inches) and 274·3 cm. in diameter (108 inches) with a capacity of 5·91 litres per millimetre and

a total capacity of 10,656 litres (2,345 gallons), thus providing a space equal to (10,656 − 8,226 =) 2,430 litres above the surface of the liquid for the ebullition of wort when it is agitated with air to a uniform density.

The depth of wort in the store tank equals (8,226 litres divided by 5·91) 1,392 mm., and this is allowed to flow continuously into the fermenter for eleven hours at a rate carefully adjusted to deliver a definite volume of wort, increasing empirically each hour with the progress of the fermentation. These differential volumes were previously calculated to provide nourishment for a logarithmic increase in the yeast, but in a series of test brews it was found that yeast cell multiplication did not conform to this theory, and by test and trial it was established that the percentages of the total volume of wort shown in the table below produced the best results.

HOURLY VOLUMES OF STORE WORT ADDED TO THE FERMENTATION

Hour.	Percentage of Store Wort (8,226 litres).	Litres of Wort.	Millimetres on Level Indicator.
1	5·0	411	69
2	5·5	452	76
3	6·0	494	83
4	7·5	617	104
5	9·0	740	125
6	10·5	864	146
7	12·0	987	167
8	13·0	1,070	182
9	13·0	1,070	182
10	13·5	1,110	189
11	5·0	411	69
	100·0	8,226	1,392

The wort is discharged direct into the fermenter from the store tank in a continuous stream and at such a rate of flow that the exact volume of wort indicated is delivered each hour. The electric level indicator is the most efficient guide to the rate of flow, or, alternatively, the store tank may be

fitted with a mechanical level indicator consisting of a flat-bottomed ball float attached to a chain which passes through a small orifice in the tank cover and runs freely over pulleys without chafing at any point. The free end of the chain is fastened to a counterpoise sliding between two boards, forming a level indicator, placed within the clear view of the attendant controlling the fermentation. The counterpoise is so shaped that a pointer sharply defines the remaining volume of wort on a graduated scale attached to the level indicator.

The indicator is graduated in millimetres on a strip of thin sheet metal, or other non-expanding material, by means of a steel measuring tape; the hourly intervals representing the specified volumes are clearly defined and marked with their corresponding numbers, and each interval is again divided into four fifteen-minute periods. The graduated strip is then securely attached to the indicator board in such a position that when the counterpoise indicates a dip of 1,392 mm. of wort there are 1,392 mm. of wort in the tank, and that zero is not indicated before the tank is empty.

In the interval occupied in charging the wort store tank the fermenter is cleaned, inspected, steam-sterilized with formalin for thirty minutes and is not reopened any more than necessary until the starting wort is protected by the addition of the seed yeast.

As soon as the store tank is charged with the required volume of strong wort the delivery pipe from the wort cooler is disconnected and the flow of wort diverted to the fermenter. At this point the density of the filtrate is falling rapidly and the gravity of the first wort flowing into the fermenter is about 4° Balling. While this vessel is filling to the 10,968 litre mark, the wort store tank, inorganic nitrogen solutions and seed yeast receive attention.

The wort in the store tank is agitated with a gentle current of air until samples taken at intervals from the discharge cock show that the gravity is constant. The gravity Balling is then determined, noted on the ferment chart and the samples discarded. In agitating this wort the counterpoise on the level indicator is a guide to the intensity of the aeration.

Violent agitation is to be avoided as it may cause surging, and thus draw dust-laden air currents into the tank to infect the wort.

Two solutions of inorganic nitrogen are required during the fermentation. A dilute solution of aqueous ammonia in a sealed iron drum, and a filtered solution of ammonium sulphate in a lead-lined container, are placed in a convenient position on the floor, or platform, from which the fermentation is controlled. Vessels of ample capacity stocked with amounts in excess of immediate requirements are necessary; a deficiency during fermentation would cause an abrupt termination of the brew to avoid producing a nitrogen poor yeast.

Preparation of Inorganic Nitrogen. Although quite unnecessary in routine practice, the approximate amount of nitrogen required for each brew may be estimated from the nitrogen content of the anticipated yield of yeast by deducting the assimilable nitrogen dissolved from the grain used in brewing; 2,240 lb. of yeast containing 2·3% nitrogen = 51·52 lb. of nitrogen consumed and retained by the yeast.

The grain used in the example mash contains the following averaged amounts of protein.

Material.	Pounds Mashed.	Protein. Per cent.	Total. Protein.
Wheat . .	604·8	12·0	72·5 lb.
Barley . .	453·6	11·2	50·8 ,,
Culms . .	362·9	25·0	80·7 ,,
Tapioca . .	1602·7	0·7	11·2 ,,

Total protein in the brew : 215·2 lb.

And this protein is expressed as nitrogen when divided by the protein factor : 215·2 ÷ 6·25 = 34·4 lb. of nitrogen. The native proteins are by no means completely dissolved from the grains in brewing and a considerable proportion remains in the spent grain, varying in amount according to the composition of the brew. Five typical examples of the analyses of

the spent grain dry substance from differential brews are shown below.

	1.	2.	3.	4.	5.
Carbohydrates	28·71	30·32	28·47	32·42	29·90
Protein. .	16·62	18·14	17·55	19·15	17·38
Fibre . .	35·45	32·63	33·60	32·94	31·33
Undetermined	19·22	18·91	20·38	15·49	21·39

The dry substance thus contains an average of 17·77% protein. In order to estimate the dry substance in the example brew it will be necessary to add the original moisture contained in the raw material to the extract dissolved out of the grain in brewing; and by subtracting this sum from the original weight of material used, the approximate weight of the spent grain dry substance is obtained.

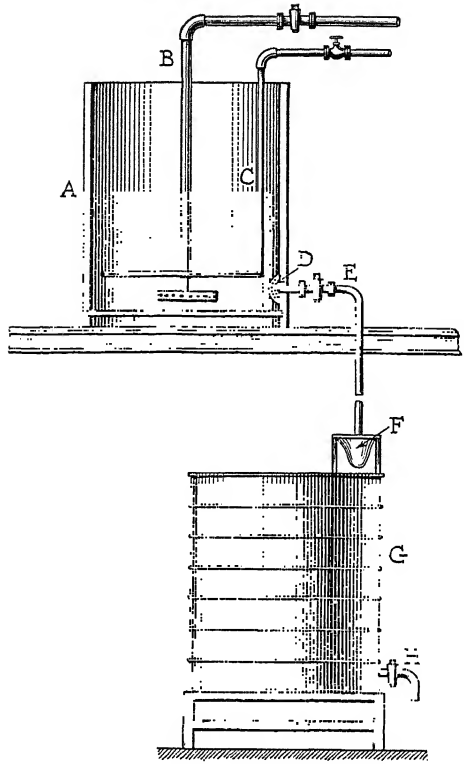
Material.	Pounds Mashed.	Moisture. Per cent.	Pounds of Water.
Wheat . .	604·8	12·0	72·5
Barley . .	453·6	11·0	49·9
Culms . .	362·9	10·0	36·2
Tapioca . .	1602·7	10·0	160·2

Total moisture in the raw material = 318·8 lb.

The extract obtained from a brew of the above composition averages 70% or 2,116·8 lb., then 318·8 + 2116·8 = 2,435 lb. of moisture and extract combined are removed from the original grains, leaving 588·4 lb. of dry substance with a protein content of 17·77% or 104·5 lb. of protein, and this divided by the protein factor = 16·72 lb. of nitrogen.

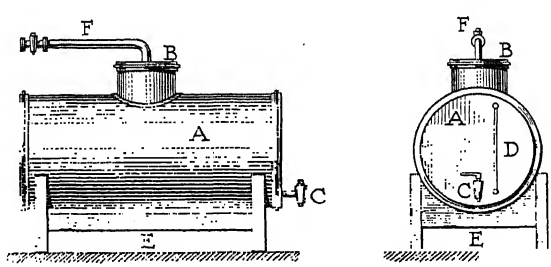
A further loss of nitrogen occurs in the spent wort as shown by the formalin number 0·15 in 19,243 litres (4,235 gallons) at the end of the fermentation, then 42,350 lb. of wort × 0·15 × 0·0014 = 8·9 lb. of nitrogen.

The approximate weight of inorganic nitrogen required for the brew is shown as follows :—



(*a*) Ammonium sulphate unit.

A. Lead-lined solution vessel. B. Lead pipe from air main, for pneumatic agitation. C. Steam pipe and perforated coil. D. Perforated sheet lead strainer. E. Lead plug cock and pipe line. F. Felt filter bag. G. Lead-lined container. H. Lead plug cock.



(*b*) Ammonia drum.

A. Iron drum. B. Clamped cover. C. Iron plug cock. D. Gauge glass. E. Wooden support. F. Connection from air main for pneumatic agitation.

FIG. 5. Inorganic nitrogen supply vessels.

Total nitrogen in the raw materials .	34·40 lb.
Less nitrogen retained in the spent grain	16·72 ,,
	17·68 ,,
Less nitrogen retained in the spent wort .	8·90 ,,
Assimilable nitrogen supplied by the brew	8·78 ,,
Nitrogen in 2,240 lb. of yeast at 2·3% .	51·52 lb.
Assimilable nitrogen supplied by the brew	8·78 ,,
Total inorganic nitrogen required . .	42·74 ,,

The amounts of ammonia and of ammonium sulphate required will vary with each brew and with the type of brew. Grain brews consume a little more aqueous ammonia than ammonium sulphate; mixed grain and molasses brews are somewhat uncertain in their consumption of these compounds, but as a rule they require nearly equal quantities of each. An all-molasses brew is supplied with nitrogen in the form of ammonium sulphate so that the sulphuric acid liberated in the dissociation of this salt may neutralize the alkalinity of the molasses store wort. In order to maintain the optimum acidities in the brew it is necessary to supplement this acid by the addition of free sulphuric acid at certain periods of the fermentation.

The exact amount of each solution consumed by any type of brew cannot be estimated in advance with any degree of accuracy and it is advisable to provide a liberal excess. In the example grain brew more ammonia than ammonium sulphate will probably be consumed, but 30 lb. of nitrogen in each compound is provided ready for use before the fermentation commences.

The aqueous ammonia is purchased in iron drums as a 25% solution (S.G. 0·915), but owing to its volatility this is far too pungent for direct use and to avoid loss it is diluted to a convenient strength. The amount required, 36·5 lb. of ammonia or 146 lb. of the 25% solution, is syphoned or forced by air pressure from the stock drum into the dilute ammonia drum and there diluted with three times its weight of water. After mixing the solution by aeration or agitation, its strength is

determined by titration against N/1 NaOH, using methyl orange indicator. Five litres of the dilute solution should then contain 1·25 litres of 25% ammonia solution.

To supply 30 lb. of nitrogen in the form of ammonium sulphate requires 141·5 lb. of the salt. This amount is weighed, placed in a sack and dissolved by suspending the sack in water. The solution is then diluted to a volume corresponding to three times the weight of the salt, 42·5 gallons or 193 litres, and filtered through a felt filter bag before use to remove the debris which the commercial salt invariably contains. The solution is kept in barrels lined with sheet lead, or in suitable anti-corrosive vessels and its strength is determined by the formalin titration. About 3 to 5 c.c. of the solution is accurately measured in a beaker with phenolphthalein and titrated with N/1 NaOH to a bright pink colour. Ten c.c. of neutral formalin is added and the solution titrated back to the same bright tint with N/1 NaOH. One c.c. of N/1 NaOH equals 0·017 gm. of NH_3 or 0·014 gm. of nitrogen.

Wooden buckets with rope handles and graduated to measure 2·5, 5, 7·5 and 10 litres are used in measuring and transferring the solutions from the containers to the fermenter. As soon as the routine determinations are completed each hour, the solutions are added to the fermentation in the quantities indicated by the tests. No ill effects follow the addition of the solutions in bulk, probably because the turbulent aeration effects an immediate dispersion, but if it is desired to avoid any possibility of local action a tub is installed at the top of the fermenter, fitted with a wood faucet set to deliver the solutions in a reasonable period, and to avoid any loss of the volatile ammonia a rubber tube slipped over the faucet delivers the solution below the surface of the fermenting wort.

The addition of phosphate to a grain brew is unnecessary unless tapioca alone is used to supply the raw starch; in this event the phosphate may be applied in the form of ammonium phosphate, or in a solution prepared from superphosphate as directed for an all-molasses brew.

Seed yeast in an amount equivalent to 12·5% of the weight of the raw material is used to inoculate the brew. This is

added to the fermenter by the simple method of breaking the seed yeast direct from the cans into the starting wort after the addition of ammonium sulphate. To avoid haulage this method is sometimes varied by dissolving the yeast in water and pumping the mixture into the fermenter, but this procedure is not recommended as the risk of infection is increased without considering the time lost in sterilizing and dismantling the pump and pipe lines.

CHAPTER IV

THE DIFFERENTIAL FERMENTATION OF A GRAIN BREW

In compressed yeast manufacture the term "differential" is often used to indicate non-alcoholic fermentation processes that aim to maintain a uniform concentration of nourishment in the fermenting wort by the addition of concentrated nourishment, in the form of wort of higher density, to compensate for the food abstracted from the dilute wort by the growing yeast. Theoretically the principle appears to present ideal conditions for yeast cultivation, but, on the commercial scale, it is found impracticable to maintain the concentration of carbohydrate nutriment at any figure above zero; the sugar is consumed almost as rapidly as it is fed, as strong wort, into the fermentation and the nitrogenous constituents of the wort varies within wide limits without exercising any influence on the fermentation.

Assuming for instance that it is desired to maintain the nourishment at a uniform concentration of 2% of the fermenting wort, with the sugar nitrogen and mineral matter in suitable proportions; the difficulty of estimating the sugar content of the wort from time to time with sufficient rapidity for the determination to be of any service in maintaining the equality of concentration of this substance is complicated by the fact that wort contains other optically active and reducing substances besides sugar.

The theory of the differential principle of yeast nourishment was first applied, with indifferent results, in efforts to obtain higher yields of yeast and alcohol from strong spirit brews, but eventually the increasing demand for compressed yeast exceeded production from fermented distillery and vinegar brewery worts and the principle was combined with Pasteur's discovery of the effect of abundant aeration upon fermenting

yeast and applied to the production of yeast from extremely dilute worts. The term " differential " thus lost its original fermentative significance but survived to indicate the evolution of the process.

In nature, unrestricted yeast growth is prevented by a combination of natural forces, otherwise if each yeast cell produced only one daughter cell, under optimum conditions and with hourly buddings, over seventeen million cells would be produced from one single yeast cell in twenty-four hours.

Numbers of this order cannot be obtained in practice owing to environmental limitations, but in the differential process the conditions under which the yeast is cultivated are so arranged that unrestricted cell multiplication is stimulated to produce yields of yeast hitherto unknown.

In differential brews fermented under identical conditions, the yield of yeast depends upon the amount of sugar supplied to the yeast. In the following table the rate of yeast growth in a differential brew containing 48% of sugar, was determined by hourly microscopic cell counts with a hæmocytometer and shows a constant increase in the number of cells from the

RATE OF YEAST GROWTH

Time.	Litres of Strong Wort added.	Total Volume in Fermenter in litres.	Actual Count of Yeast Cells.	Relative Increase for the Total Volume of Wort.	Ratio of Cell Increase per Hour.
5 p.m.	—	20,000	18	—	—
6 ,,	started	20,000	22	—	1·22
7 ,,	750	20,750	26	26·97	1·22
8 ,,	750	21,500	32	33·18	1·23
9 ,,	750	22,250	38	39·32	1·18
10 ,,	900	23,150	44	45·77	1·16
11 ,,	1,050	24,200	52	54·35	1·18
12 ,,	1,100	25,300	63	65·86	1·21
1 a.m.	1,450	26,750	74	78·24	1·18
2 ,,	1,500	28,250	87	91·87	1·17
3 ,,	1,500	29,750	103	108·46	1·18
4 ,,	1,500	31,250	120	126·05	1·16
5 ,,	750	32,000	127	130·04	1·03
6 ,,	—	32,000	127	—	—
7 ,,	—	32,000	127	—	—

commencement of the fermentation, with a slight rise or fall in the rate each hour, as long as the yeast was supplied with food until its conclusion.

The most important points to be observed in controlling the differential fermentation may be indicated by briefly describing the main factors that contribute to its successful application.

Sugar. Yeast obtains the energy necessary for growth and reproduction from the dissociation of sugar. That part of the carbon content of the sugar transformed into alcohol in a feebly aerated fermentation is, in a differential brew, utilized by the yeast to construct new cell substance but the same volume of carbon dioxide is formed in each case. Sugar is the chief constituent of the wort prepared for yeast production, and the manner in which it is supplied to the fermenting yeast is one of the important features of the differential process.

Although the amount of sugar in the starting wort is very small it is usually more than enough to supply the requirements of the almost dormant seed yeast for the first hour of fermentation. The flow of strong wort is then proportioned to provide the energy and food necessary for the yeast to build fresh structure and to repair the wastage of its own cell material.

The decomposition of sugar is the function of specific enzymes and as these act independently of the cell that produces them the sugar is immediately dissociated as it is supplied to the brew. A deficiency of sugar results in a partial suspension of reproductive activity and an excess involves a waste of material represented by the formation of alcohol that is too dilute to be recovered commercially and is dissipated during the later stages of the fermentation.

For the production of consistently high yields of yeast it is essential to reserve at least 95% of the extract as strong wort in the store tank and to supply this in a continuous stream carefully regulated to deliver the exact volumes indicated on the ferment chart. The rate of the differential flow and the volume of air increases as the fermentation progresses towards completion and then instead of ceasing abruptly, reduced volumes of both air and wort are supplied to the brew for one hour to allow the immature cells to complete their growth.

Gravity Balling. The density of the fermenting wort is determined without filtration by means of the Balling saccharometer. The initial gravity of the starting wort may vary between 0·25° and 0·5° Balling and, owing to the uniform increase in the amount of yeast formed after the addition of seed yeast, rises progressively to between 2·5° and 3·0° Balling at the end of fermentation.

Nitrogenous Nutriment. Diffusable nitrogen is essential to yeast growth, and to produce the exceptionally heavy yields of yeast characteristic of the differential process there must be an adequate supply of assimilable nitrogen available to the yeast throughout the whole period of fermentation. At the beginning this may amount to, or exceed, 0·0126% due to the presence of organic nitrogen compounds derived from the grain plus inorganic nitrogen added to the starting wort in the fermenter prior to the addition of seed yeast.

The assimilable organic nitrogen and that from the initial charge of ammonium sulphate is rapidly consumed and, three hours later, it is necessary to supplement the comparatively meagre supply of diffusable nitrogen in the strong wort by the addition of inorganic nitrogen in the form of NH_4OH and $(NH_4)_2SO_4$ and to continue these additions at intervals of one hour, in quantities that vary with the requirements of the yeast, to maintain the nitrogen concentration continuously above 0·0042% (formalin number 0·3) until four hours before the end of the fermentation.

The addition of inorganic nitrogen then ceases and its concentration in the wort is allowed to decline through yeast activity to below 0·0028% (formalin number 0·2). A nitrogen concentration exceeding this value at the end of fermentation is quite unnecessary and the surplus represents so much wasted material. The amount of assimilable nitrogen in the fermenting wort is quickly estimated by Sorensen's formalin titration and is reported as the formalin number.

Formalin Number. The formalin number is determined by neutralizing 100 c.c. of the unfiltered wort with N/1 NaOH to a bright pink colour with phenolphthalein indicator ; 10 c.c. of neutral 40% formalin solution are then added and the formalin number is defined as the number of cubic centimetres

of N/1 NaOH required to restore the colour of the wort to the former pink tint. One cubic centimetre of N/1 NaOH = 0·014 gm. of nitrogen in 100 c.c. of wort. The amount of nitrogen consumed by the yeast is indicated by the formalin number, and the addition of inorganic nitrogen to the brew is regulated to maintain the formalin number continuously above 0·2 during the period of active fermentation. Nitrogen should not be added when the formalin number is high (0·6 or over) or when it indicates little or no consumption during the preceding hour.

Inorganic Nitrogen. As 1 kg. of $(NH_4)_2SO_4$ contains 25·83% of NH_3 and 1 litre of 25% ammonia solution contains 25% NH_3 the nutritive value of the two compounds is approximately the same. For the purpose of estimating the inorganic nitrogen required, or used, during the fermentation of a differential brew it is the practice to assume that 25% of the total amount, litres as kilos, represents the NH_3 consumed and the nitrogen amounts to 14/17 of this percentage.

Hydrogen Ion Concentration. In common with other biological processes yeast growth depends upon the maintenance of a strictly limited hydrogen-ion concentration in its environment, and it is due to a recognition of this fact and to the development of rapid methods for the determination of *p*H values in fermenting wort that made the differential process a commercial possibility.

In spirit fermentations the lactic acid present in the wort is derived solely from products of grain constituents through the activity of lactic acid bacteria. Yeast is indifferent to wide variations in the concentration of this feebly ionized acid, the buffer action of which prevents any effective change in the *p*H of the fermenting wort.

In a differential fermentation the conditions are quite different, the organic acids are rapidly dissipated and three hours after the commencement of the fermentation the acidity consists of sulphuric acid dissociated from the ammonium sulphate. This acid is highly ionized and if the hydrogen ion concentration (*p*H) is not carefully controlled by the addition of ammonium hydrate, conditions quite unfavourable to yeast growth may rapidly develop. *p*H is determined in the starting

wort before the addition of $(NH_4)_2SO_4$ and seed yeast and every hour thereafter until the conclusion of the fermentation. From the third and at intervals of one hour during the following seven hours the addition of ammonia and ammonium sulphate is proportioned so that *p*H is maintained as closely as possible on *p*H 3.

The *p*H determination together with the acid titration indicates the form in which the ammonia is to be added to the brew. For instance, if the titration acidity is falling and *p*H is rising the amount of $(NH_4)_2SO_4$ solution added to the brew must be proportionately greater than that of NH_3 to restore the optimum acidities, and the total amount that should be added is indicated by the consumption of nitrogen during the preceding hour as shown by comparing the formalin numbers covering the interval.

Acidity. The titration acidity of fermenting wort generally varies with *p*H so that the acid value is rising while the numerical value of *p*H is falling. The acidity of the starting wort is usually about 0·3° but owing to the production of inorganic acids during the fermentation and the accumulation of acid phosphates from the strong wort, the titration acidity increases progressively until at the end of the fermentation it amounts to between 1·5 and 2·0° acidity.

The acid is determined in 100 c.c. of the unfiltered wort by titration with N/1 NaOH using neutral litmus paper indicator, each cubic centimetre of N/1 NaOH solution required to neutralize the acid is termed a brewer's degree of acidity.

Aeration. The essential object in aerating a differential brew is to keep the wort continually saturated with dissolved oxygen. The production of alcohol and a correspondingly reduced yield of yeast follows any reduction in the air volumes necessary to achieve this object, but an excess of air simply involves a waste of power. At the start of fermentation the amount of yeast present in the wort is relatively small and the consumption of oxygen is in the same ratio but the yeast rapidly multiplies as the quantity of strong wort, rich in food, increases with the progress of the fermentation and the volumes of air injected into the brew are periodically increased until the maximum is supplied.

THE DIFFERENTIAL FERMENTATION

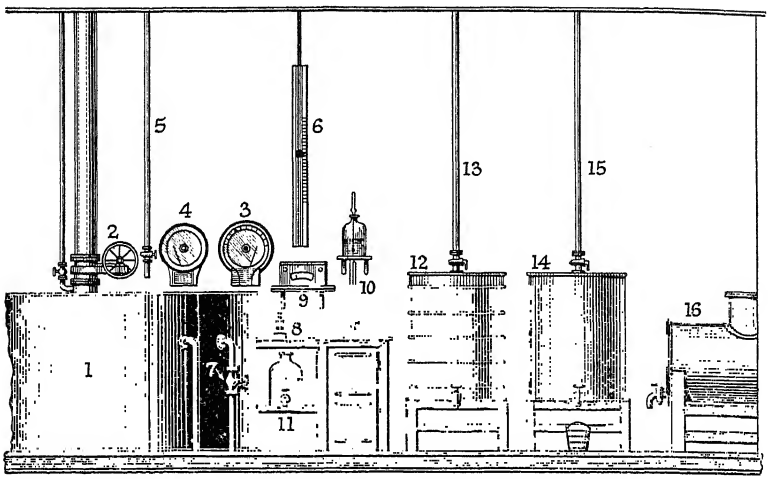


FIG. 6. Equipment of the fermentation Control Room.

1. The upper portion of the fermenter. 2. Air main and control valve. 3. Recording air volume meter. 4. Recording thermometer. 5. Store wort supply pipe and control valve. 6. Wort store tank level indicator. 7. Cooling coil control valve. 8. Microscope. 9. Potentiometer, for *p*H determinations. 10. Burette and normal soda solution. 11. Sulphuric acid. 12. Filtered ammonium sulphate vessel. 13. Lead pipe from ammonium sulphate solution vessel. 14. Phosphate solution container. 15. Syphon pipe from phosphate solution vessel. 16. Aqueous ammonia container.

Theoretically 1·066 kg. (0·764 cubic metre) of oxygen is necessary to oxidize 1 kg. of sugar but only a small proportion of the oxygen blown into the wort is utilized, the percentage being governed primarily by the interval of contact and in a lesser degree by the temperature and progress of the fermentation.

More oxygen is absorbed from a given volume of air in a deep fermentation than in a shallow one, owing to the longer time the injected air takes to reach the surface; and absorption is accelerated as the increasing temperatures approach the optimum for yeast activity. The dimensions of the fermenter therefore becomes a feature of prime importance when depth of wort is considered in its relation to economical aeration; and, irrespective of the size of the brew, the fermenter is designed so that the final dip of the fermenting wort is not less than 3 metres (118·2 inches).

In a fermentation shallower than this, more air must be injected, determined by test and trial, in an effort to obtain the same quality and yield of yeast, but as yeast can only assimilate oxygen that is dissolved in the wort the result becomes more uncertain as the dip becomes shallower. Under the optimum conditions for this process the total volume of oxygen injected into the brew amounts to about seven times the theoretical and with the CO_2 evolved results in an exceptionally turbulent aeration with the formation of voluminous froth.

By referring to the directions given on the differential ferment chart it will be observed that the volumes of air injected into the brew increase empirically from the start as the volume of the flow of strong wort increases, until 1·3 cubic metres of air per hour per kilogram of material mashed is delivered. At the end of fermentation only sufficient air is injected to keep the yeast in suspension until it is separated from the wort. The volumes of air necessary for the example brew are shown in the following tables as cubic metres per hour and in the English equivalent, cubic feet per minute.

Period in Hours.	Cubic Metres of Air per Kilogram of Raw Material per Hour.	Kilograms of Material Mashed.	Total Cubic Metres of Air injected into the Brew per Hour.
1	0·625	1,371	857
4	1·000	,,	1,371
7	1·300	,,	1,782
1	0·625	,,	857

One cubic metre of air per hour per kilogram of material when converted to the English equivalent equals 0·26741 cubic foot of air per minute per pound of raw material.

Period in Hours.	Cubic Foot of Air per Minute per Pound of Raw Material.	Pounds of Raw Material Mashed.	Total Cubic Feet of Air injected into the Brew per Minute.
1	0·16513	3,016	498
4	0·26741	,,	806
7	0·34763	,,	1·048
1	0·16513	,,	498

Temperature. The activity of yeast is almost entirely dependent upon the temperature, reproduction and fermentation increasing as the temperature rises to the optimum at 30° C. In a differential fermentation the contributing factors, temperature, aeration, amount of seed yeast, *p*H, nitrogen concentration and strong wort additions are all balanced to provide the optimum conditions for the unrestricted multiplication of yeast cells. Temperature control is thus so closely related to the principles governing the process that any departure from the temperatures specified on the ferment chart disturbs the above fermentation equilibrium and not only dislocates the routine of the brew but may affect the quality of the product.

The amount of heat liberated by the oxidation of sugar amounts to 674 calories from 1 gm. molecule (180 gm.) but in a differential fermentation the heat liberated by the oxidation of the sugar produced from 1,371 kg. of raw material is supplemented by the heat generated in compressing the air to over 4 lb. per square inch and, although a considerable amount of heat is dissipated by surface evaporation and radiation, the temperature of the brew is only controlled by passing chilled water through cooling coils, immersed in the wort, in a volume corresponding to 3 litres of water per hour per kilogram of raw material mashed; the water entering the coils at 10° C. and issuing at 25° C. In a 1,371 kg. brew this amounts to an average of 4,113 litres, or 900 gallons of chilled water per hour.

Fermentation commences at 78·6° F. (26° C.) and the temperature requires little control for the first two hours, but as the differential flow of strong wort increases the heat developed by the activity of the yeast increases with the progress of the fermentation, the temperature is then carefully controlled and maintained at 78·6 ° F. for the first six hours, it is then allowed to rise at a uniform rate until it reaches 86° F. during the last hour of the flow of strong wort.

Seed Yeast. Under identical conditions, as regards nutrition temperature and aeration, the rate of cell reproduction is directly proportional to the amount of seed yeast present in

the wort, therefore sufficient seed yeast must be added to the brew to consume the sugar in the starting wort before the differential flow of strong wort commences.

If the amount of seed yeast is insufficient to convert the full quota of sugar into cell substance material is wasted by the formation of alcohol and this wastage continues during the fermentation for a period that depends upon the yeast deficiency. To add seed yeast in excess of the quantity necessary to consume the sugar simply reduces the amount of food and energy shared by the individual cells and their activity is correspondingly reduced, the yield of yeast is not affected and the quality remains normal.

Fermentation Control. The development of the growing yeast is closely followed by frequent microscopic observations and the fermentation controlled according to the above instructions and the details recorded on the specimen ferment chart. The first row of figures on the fermentation record are

FERMENT CHART

DATE..... BREW No..... GENERATION 12. FERMENTED by.....

RAW MATERIALS.		CHEMICALS.		MASHING AND SOURING.					FILTRATION.
	Kilo-grams.		Kilo-grams.	Time.	Mash Acidity.	Gravity Balling.	Tempera-ture deg. Fahr.	Mash heated to	
Maize .	—	Lime	—	10 a.m.	—	—	145	—	
Wheat .	—	$Al_2(SO_4)_3$	—	1 p.m.	0·5	16·7	132·5	—	Started, 10 a.m.
Barley .	205	$(NH_4)_2SO_4$	28	6 p.m.	1·2	—	131·5	138	Finished, 3 p.m.
Tapioca .	728	NH_3	24	10 p.m.	Agitated.	—	—	—	Hours, 5
Molasses .	—	H_2SO_4	—	2 a.m.	,,	—	—	—	Mashed, 1 p.m.
Wheat Malt	274	$CaH_4(PO_4)_2$	—	6 a.m.	,,				
Chev. Malt	—	—	—	9 a.m.	7·0	16·5	135	168	
Cape Malt									
Culms .	164								
Total .	1,371								

test results and observations of the starting wort before the addition of ammonium sulphate and seed yeast, the succeeding figures in each column clearly show the progress of the fermentation until its conclusion. The yield of yeast is shown and the notes detail the properties of the yeast that the brew produced. The ferment chart with its records of the mashing,

Fermentation Record

Time in Hours.	Temp. deg. Fahr.	Gravity deg. Balling.	Deg. Acidity	pH	Formalin Number	Cubic Feet Air per Minute.	Litres of Sterile Wort added	Litres NH_3.	Kilograms $(NH_4)_2SO_4$	Kilograms H_2SO_4	Litres $CaH_4(PO_4)_2$ Solution.
5 p.m.	78·5	0·3	0·3	4·5	0·1	498	—	—	10·0		
6 ,,	78·5	0·85	0·4	4·0	0·9	806	Start.				
7 ,,	78·5	1·1	0·6	3·6	0·6	806	411				
8 ,,	78·5	1·3	0·9	3·2	0·26	806	452	2·5	2·5		
9 ,,	78·5	1·55	1·2	3·0	0·4	806	494	2·0	2·0		
10 ,,	78·5	1·75	1·3	3·1	0·35	1,048	617	2·0	2·5		
11 ,,	79·0	1·9	1·4	3·0	0·3	1,048	740	3·0	2·0		
12 ,,	80	2·1	1·5	3·0	0·35	1,048	864	3·0	2·0		
1 a.m.	82	2·25	1·6	3·2	0·3	1,048	987	2·5	3·0		
2 ,,	83	2·4	1·7	2·8	0·35	1,048	1,070	4·0	2·0		
3 ,,	84	2·6	1·7	2·8	0·35	1,048	1,070	5·0	2·0		
4 ,,	86	2·75	1·8	3·0	0·25	1,048	1,110				
5 ,,	86	2·8	1·8	3·2	0·2	498	411				
6 ,,	86	2·8	1·75	3·4	0·15	Feeble.					
Separated Wort 1·2							Total	24	28		

Extract, Yields and Notes

Store wort, 8,226, litres 11·7 gravity Balling. 962·44 Balling hectolitres.
Start wort 10,968, litres 0·3 gravity Balling. 32·9 Balling hectolitres.
Sugar 50·4%. Total 995·34 Balling hectolitres = 72·5% Extract.

Yield of yeast.
Kgs. of yeast gross, 1,337.
Kgs. of seed yeast, 171·8 brew No. Gen.
Kgs. of yeast nett, 1,165·2 = 84·9%.

Dough test, 50 minutes.
Sugar test, 825 c.c.
Protein, 52·8%.

Calculated yield of yeast.
Wort + yeast 2·8° Balling = 1·0112 specific gravity.
Wort − yeast 1·2° Balling = 1·0048 specific gravity.
Difference in specific gravity = 64 = kilograms of yeast 1,339 gross.

acidification, filtration and fermentation thus constitutes a permanent record with all the information necessary for future comparison or repetition.

The Commencement of the Fermentation

As soon as the fermenter is charged with the required volume of starting wort, 10,968 litres, sufficient air is injected to agitate the wort and, if necessary, either steam is admitted through the air main or chilled water through the cooling coils to adjust the temperature of the starting wort at 78·6° F. A sample of the wort is removed for the routine tests and the analytical details are noted on the fermentation record under the specified headings. Ten kilograms of ammonium sulphate

are then added to the starting wort followed by 171·8 kg. of seed yeast and the fermentation commences immediately with the injection of 498 cubic feet of air per minute for the first sixty minutes.

First Hour. After sixty minutes' fermentation the volume of air is increased to 806 cubic feet per minute and the differential flow of strong wort from the store tank to the fermenter is started and regulated by setting the indicator on the discharge cock in such a position that the wort flows into the fermenter at the rate of 411 litres per hour. The correct adjustment of the tap is readily determined by a simple calculation after measuring the rate of flow of strong wort with a litre cylinder and a stop watch.

A sample of the fermenting wort is then drawn from the fermenter and the gravity Balling, acidity and formalin number determined in the unfiltered wort. Time is saved by doing this while 50 or 60 c.c. of the wort is filtering through a ribbed filter paper for the colorimetric determination of *p*H. The temperature of the fermentation is observed and with the details of the routine tests immediately noted on the fermentation record.

In the example, gravity Balling shows an apparent increase to 0·85° Balling; this is largely due to the seed yeast that was added after the first reading and in a smaller measure to subsequent yeast cell multiplication. The temperature of the fermentation is maintained at 78·6° F. without difficulty during this interval because the heat developed by fermentation is proportional to the amount of yeast present in the wort and at this period the quantity is relatively small. The acidity has increased to 0·4°, indicating the dissociation of ammonium sulphate and lactic acid, and this is confirmed by an increase in the hydrogen ion concentration to *p*H 4·0. The formalin number 0·9 is normal for this period of the fermentation and confirms the presence of an adequate supply of assimilable nitrogen to nourish the yeast for the specified interval, three hours after the start of the fermentation, before any more inorganic nitrogen is supplied.

The increased volume of air injected into the wort promotes the formation of immense volumes of froth which, if not

dispelled with a spray of melted fat, rises with incredible rapidity and overflows the tank. The yeast is microscopically examined at frequent intervals and presents an appearance similar to one of the accompanying illustrations.

Second Hour. The differential flow of strong wort from the store tank is increased to 452 litres per hour. The volume of air injected into the wort remains the same and the temperature is maintained at 78·6° F.

A sample of the fermenting wort is taken for examination and the tests show that yeast growth has advanced the gravity Balling to 1·1°. Acidity has increased to 0·6° and the hydrogen ion concentration to *p*H 3·6. Although nitrogen consumption has reduced the formalin number to 0·6 the brew contains sufficient assimilable nitrogen to supply the requirements of the yeast during the next hour without reducing the formalin number below the permissible minimum.

Third Hour. The differential flow of strong wort is increased to 494 litres per hour, the temperature is observed and a sample of the fermenting wort drawn for the routine determinations. A rising temperature, attributable to the activity of the increasing amount of yeast in the fermentation as indicated by a rise in the gravity of the wort to 1·3° Balling, is controlled by admitting chilled water to the cooling coils. The titration acidity has increased to 0·9 ° and the hydrogen ion concentration to *p*H 3·2. Nitrogen consumption has reduced the formalin number to 0·26 and as this is below the optimum (0·3) inorganic nitrogen is added to the fermenter without delay.

The amount of nitrogen required is indicated by its consumption during the preceding hour and may be estimated by a comparison of the formalin numbers covering the interval, but a more accurate forecast is possible by estimating the amount of nitrogen that will be consumed by the yeast plus the amount necessary to ensure a concentration equivalent to formalin number 0·3. From this sum the assimilable nitrogen content of the fermenting wort and of the periodical volume of strong wort is deducted and the difference is calculated back to show the required volume of 25% inorganic nitrogen solution.

		Grams of Nitrogen
In a differential fermentation yeast increases at the rate of 1·2 times per hour, therefore the anticipated yeast increase for the period under review amounts to 59 kg. with a nitrogen content of 2.0% representing 1.180 gm. of nitrogen		1,180
The volume of wort in the fermenter will then amount to 12,325 litres at formalin number 0·3 (0·3 × 14 mg. N. per 100 c.c.)	=	517
Total nitrogen required in grams	=	1,697
In the fermenter there are 11,831 litres of wort containing 36·4 milligrams of nitrogen per litre (formalin number 0·26) = 430 gm. N.	=	430
In the 494 litres of strong wort there are 405 gm. of assimilable nitrogen	=	405
Grams of nitrogen in the wort		835

The deficiency thus amounts to 1,697 — 835 = 862 gm. of nitrogen representing 4·2 litres, or kilograms, of 25% ammonia solution, but as a slight surplus is desirable 5 litres, or kilos, of ammonium compounds are immediately added to the fermenting wort. In routine operations it will be found quite unnecessary to calculate the amount of nitrogen required on each occasion as experience will soon indicate the necessary amount.

The form in which the nitrogen should be applied is indicated mainly by *p*H and in a lesser degree by titration acidity. In worts produced from grain, yeast is most active at *p*H 3·0 and in this particular instance it is apparent that if 5 kg. of ammonium sulphate are added to the brew the hydrogen ion concentration will be increased to below *p*H 3·0 owing to the effect of the sulphuric acid dissociated from the ammonium sulphate, or if the equivalent of aqueous ammonia is used some of it will combine with the free sulphuric acid

already in the brew and by reducing the titration acidity will reduce the hydrogen ion concentration to above *p*H 3·0. By adding approximately equal quantities of ammoniacal compound both *p*H and titration acidity will be maintained within the desired limits. Of the fully diluted aqueous ammonia solution 10 litres (= 2·5 litres 25% NH_3) and 7·5 litres of ammonium sulphate solution (= 2·5 kilos $(NH_4)_2SO_4$) are either added direct to the brew or placed in a tub at the top of the fermenter from which it gravitates through a tube and is delivered at a point below the surface of the fermenting wort.

Fourth Hour. The differential flow of strong wort is increased to deliver 617 litres of wort per hour, but the air volume remains the same at 806 cubic feet per minute. The heat developed by the fermentation requires an increase in the flow of chilled water through the cooling coils to maintain the temperature at 78·6° F. An advance in the density of the fermenting wort to 1·55° Balling represents the normal increase in the amount of yeast and microscopic examination shows a field of healthy cells with buds adhering in various stages of development.

Acidity has increased to 1·2° and hydrogen ion concentration to *p*H 3·0. The consumption of nitrogen is normal but the formalin number 0·4 indicates that a little less inorganic nitrogen, the equivalent to 4 kilos, may be used than on the last occasion, while *p*H and titration acidity show that equal quantities of NH_3 and $(NH_4)_2SO_4$ will preserve the optimum acidities. Froth has practically disappeared and the surface of the fermentation has a uniformly creamy appearance with columns of mist rising to the top and flowing over the edges of the tank.

Fifth Hour. The differential flow is adjusted to deliver 740 litres of strong wort to the fermenter during the next hour, and the volume of chilled water passing through the cooling coils is increased to prevent any rise in the temperature of the fermentation. The speed of the compressor is increased to deliver 1,048 cubic feet of air per minute into the brew and that uotput remains unaltered for the next seven hours.

The routine determinations show a satisfactory increase in

the amount of yeast formed by a rise in the gravity Balling to 1·75. The acidity has increased to 1·3° but the hydrogen ion concentration is reduced to *p*H 3·1, indicating that a greater proportion of $(NH_4)_2SO_4$ is necessary to restore *p*H to the optimum value. A satisfactory concentration of assimilable nitrogen is shown by formalin number 0·35 but the chemicals will be increased to an equivalent of 4·5 kg. by the addition of 2 litres of NH_3 and 2·5 kilos. of $(NH_4)_2SO_4$ in order to provide the nourishment required for a greater increase in yeast during the interval.

Microscopic examination of the yeast at this period shows a slight change in shape, a few cells retain the spherical form while others appear to be slightly reduced in size with a small flattened area on one side. The chain formation that characterizes yeast cell multiplication in spirit worts is absent; pairs only are seen, the mother and daughter cells becoming separated by the turbulent aeration as soon as the latter reach maturity.

The differential fermentation with its abundant aeration presents ideal conditions for the rapid multiplication of all strongly aerobic micro-organisms, particularly *mycoderma* and *monilia candida* which, when present, assume an appearance peculiar to the process. The microscopic field may look quite normal with the yeast cells in pairs and budding freely until an agglomeration of yeast-like cells, which may consist of any number from fifty upwards, appears in the form of a pellet. The presence of this infection, which may rapidly assume serious proportions, indicates either the absence of bacteriological cleanliness in the plant or faulty air filters.

Sixth Hour. The differential flow is increased to deliver 864 litres of strong wort per hour and the temperature is allowed to rise to 79° F. The apparent density of the fermenting wort has increased, principally by yeast growth, to 1·9° Balling, and the increase in the proportion of ammonium sulphate supplied with the chemicals on the last occasion has effected an increase in hydrogen ion concentration to *p*H 3·0 and titration acidity to 1·4°.

Formalin number 0·3 shows the optimum concentration of nitrogen for the time being and, in an effort to repeat this

value at the next analysis, the amount of added nitrogen will be further increased to the equivalent of 5 kg., but to check any further variation in *p*H or titration acidity the proportion of ammonia will be increased to 3 litres and ammonium sulphate reduced to 2 kg.

Seventh Hour. The differential flow is further increased to deliver 987 litres of strong wort from the reservoir to the fermenter during the next hour. The temperature is allowed to rise to 80° F. and a sample of the fermenting wort is taken for the routine tests. The normal increase in the amount of yeast produced during the hour is indicated by a rise in the gravity Balling to 2·1°.

The proportion of ammonia was increased in the last addition of inorganic nitrogen in order to check a too rapid increase in titration acidity; that this was necessary is shown by the rise from 1·4° to 1·5° acidity but the hydrogen ion concentration was maintained at the optimum *p*H 3·0. Nitrogen consumption was slightly less than that anticipated as shown by the formalin number 0·35. A review of these details indicate that the same conditions may be repeated after the interval if the same amount of inorganic nitrogen is added to the brew and in the same proportions.

Eighth Hour. The flow of strong wort is increased to deliver 1,070 litres per hour and the temperature is allowed to rise to 82° F. Gravity Balling has advanced to 2·25° and the wort is so heavily charged with yeast that it is necessary to remove the yeasty film that obscures the graduations and corrections on the bulb of the saccharometer in order to obtain a correct reading. Acidity has increased to 1·6° but hydrogen ion concentration has declined to *p*H 3·2 showing that more ammonium sulphate must be supplied.

Nitrogen concentration is normal at formalin number 0·3 but as a slight surplus is desired at this stage the nitrogen additions will be advanced to the equivalent of 5·5 kg. by the addition of 2·5 litres of ammonia and 3·0 kilos of ammonium sulphate.

In the microscopic field the yeast cells appear to be much smaller in diameter than at the commencement of the fermentation but the vitality of the cells is demonstrated by their resistance to the usual staining reagents. The volume of air

injected into the brew remains the same but the turbulence of the aeration appears to increase with the increase in the depth of the fermenting wort. This is largely due to small air bubbles combining as they rise to the surface from the bottom of the tank and to the greater volumes of CO_2 evolved as the fermentation progresses.

Ninth Hour. The flow of strong wort continues at the same rate to deliver 1,070 litres per hour and the temperature is advanced to 83° F. The apparent density of the fermenting wort, 2·4° Balling, indicates the maximum rate of yeast growth. Titration acidity shows an advance to 1·7° and hydrogen ion concentration to *p*H 2·8. The latter value is high and will be corrected by increasing the supply of ammonia. Nitrogen consumption was normal during the hour and its present concentration, formalin number 0·35, will be repeated after the interval by increasing the amount of chemicals to 6 kg., using 4 litres of ammonia and 2 kilos of $(NH_4)_2SO_4$.

Tenth Hour. The strong wort flow is advanced to deliver 1,110 litres per hour, the maximum volume for the brew, and the temperature is allowed to rise to 85° F. The density of the fermenting wort now registers 2·6° Balling; acidity has increased to 1·75° and hydrogen ion concentration to *p*H 2·8, showing that the ratio of ammonia to ammonium sulphate must be further increased to restore the acidity to *p*H 3·0. The formalin titration 0·35 indicates a slight excess in the concentration of nitrogen but an examination of this result when compared with the anticipated increase in yeast during the remaining period of the fermentation shows that the equivalent of 7 kg. of 25% ammonia compound will be necessary for yeast nourishment and this, the last addition of inorganic nitrogen, is supplied as 5 litres of ammonia and 2 kg. of ammonium sulphate.

Eleventh Hour. The flow of strong wort is reduced to deliver the final volume, 411 litres, during the next hour. The attention necessary to ensure that the flow extends full period may depend upon the depth of liquor in the store tank if this vessel is fitted with a ball float, because the float is sometimes immersed in the strong wort to such a depth that it now rests upon the bottom of the tank and does not indicate the

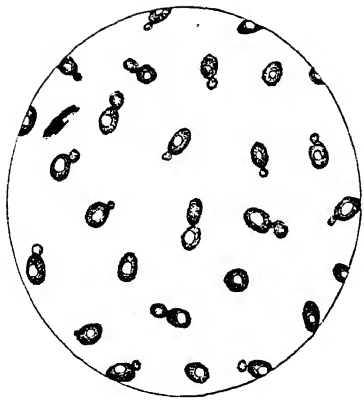
volume of wort. The temperature is allowed to rise to 86° F. Aeration is maintained at 1,048 cubic feet per minute. The apparent density of the wort has advanced to 2·75° Balling, the titration acidity to 1·8° and hydrogen ion concentration has declined to the optimum, *p*H 3·0. The formalin number 0·25 confirms the presence of sufficient nitrogen to supply the yeast with this form of nutriment for the remainder of the fermentation.

During this and the previous hour the depth of liquor in the fermenter combined with the turbulent aeration will test the accuracy with which the air distributing pipes have been levelled and anchored. Surging of the wort sometimes commences and may become so violent as to distort the tank if the distributing pipes or air jets are not truly horizontal. When surging occurs the only immediate remedy is to shut off the air, resuming aeration immediately the liquor comes to rest.

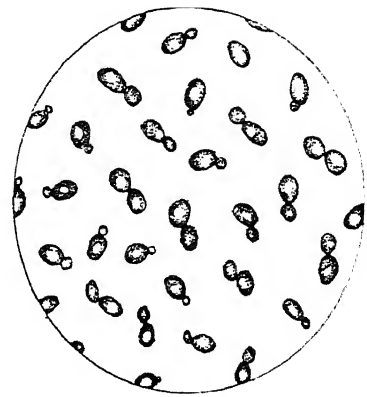
Twelfth Hour. The volume of air is reduced to 498 cubic feet per minute and the temperature is maintained at 86° F. Titration acidity has not varied from 1·8° recorded at the previous test but the hydrogen ion concentration is lower at *p*H 3·2. The continuity of nitrogen consumption is shown by the reduction in formalin number from 0·25 to 0·2 and in consequence the gravity Balling has increased to 2·8°.

The last hour of the fermentation is termed the maturing or "ripening period" during which important changes occur in the yeast, the immature buds develop and reach the adult stage and consequently the cells in general appear more uniform in size and spherical in shape. Actively fermenting yeast secretes only sufficient of the technically important zymase "alcoholase" for its immediate needs and, irrespective whether the yeast is cultivated in feebly aerated spirit worts or in the turbulently aerated differential brews, it is essential to allow this ripening period of at least one hour after fermentation ceases for the yeast to develop its maximum zymase content.

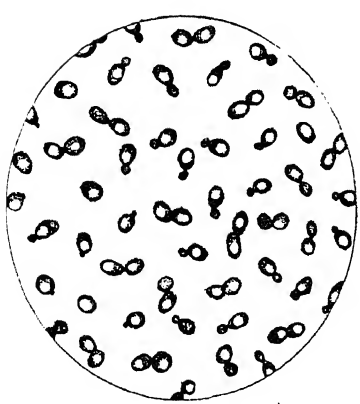
Thirteenth Hour. The air is reduced to a volume just sufficient to agitate the wort and prevent the yeast from settling on the bottom of the tank where degeneration may occur by overheating in a thick sediment of yeast. The temperature, still on 86° F., is allowed to fall whilst the yeast is being



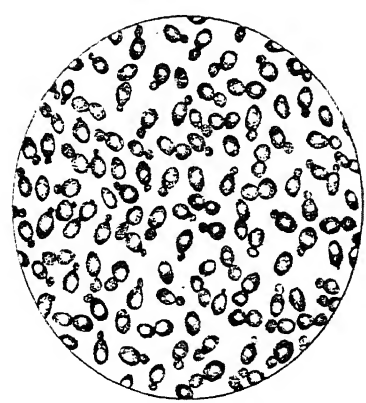
First hour of fermentation.



Third hour of fermentation.



Fifth hour of fermentation.



Seventh hour of fermentation.

FIG. 7. The microscopic appearance of differentially nourished yeast cells at intervals during growth, showing a progressive reduction in the size of the cells and their rapid increase in number.

[*To face p.* 110.

separated. The routine tests show no increase in the gravity Balling, titration acidity is lower at 1·75°, hydrogen ion concentration is reduced to *p*H 3·4 and nitrogen consumption has reduced the formalin number to 0·15. The pronounced smell of hydrogen sulphide at this period is a characteristic feature of the process.

The control of the process, with the addition of chemicals in form and amount as required by the interpretation of the routine tests, as detailed above in the example brew is commonly experienced when yeast is propagated in worts derived from grain. The ratio of ammonia to ammonium sulphate may vary slightly in different brews even when the worts are prepared from the same kind of grain and under identical conditions, but wide variation in the total amount of inorganic nitrogen consumed are possible and may be due not only to differences in the nitrogen content of the raw material, and the percentage of native proteins hydrolysed in the mashing process, but to the amount of yeast produced by the fermentation.

Yeast growth immediately ceases when any one of the essential food substances, sugar, nitrogen, or mineral matter, becomes exhausted. The supply of nitrogen and mineral matter is under control and unlimited as far as the yeast is concerned, so that the yield of yeast ultimately depends upon the percentage of sugar produced from the grain in the mashing process. The theoretical yield of yeast amounts to 90% when 50% of sugar is produced from the raw materials and, for comparative purposes, it is customary to calculate the yeast yield back to the basis of 50% sugar.

Separating and Pressing the Yeast

The fermented wort gravitates through a pipe, connected with the discharge cock on the bottom of the fermenter, to the separator feed main; or, if space does not permit a gravity feed, the wort is pumped up into a small reservoir in which a ball valve controls the pump speed and ensures a constant supply of wort to the separators. Shortly before fermentation ceases the separators are started, the separator feed bends are examined and screwed into position and the filter-press closed

and tightened in readiness for the final process of yeast recovery.

The speeds of the separators are indicated by the tachometer, a revolution counter attached to each machine; and when this shows that the unit has reached its rated velocity, the separator is primed with cold water until both yeast and wort spouts are discharging. Fermented wort is then admitted to the separator sieve and as soon as wort and yeast appear at the discharge spouts the supply of priming water is discontinued and more wort admitted until the separator is receiving its full supply.

These machines reduce the yeast to a thick cream by eliminating four-fifths of the spent wort, and as the latter is valueless it is allowed to run to waste. A separator receiving an excessive supply of fermented wort may discharge yeast with the waste liquor. The same effect is produced by choked yeast nipples on the separator bowl and to prevent the loss of yeast by a gradual development of either of these faults the flow of waste wort is constantly examined during separation with a small test tube. A sample of the spent wort taken in the tube quickly clears of bubbles and, in the absence of yeast, the wort appears bright in the bottom of the tube; but if the wort remains turbid after a few seconds' standing, yeast is probably flowing down the drain and the faulty separator is located and given immediate attention.

The yeast concentrate flowing from the discharge spout of the separator is conducted, by pumping, to flow over a vertical yeast cream cooler and is chilled to as low a temperature as possible. The activity of yeast is entirely dependent upon the temperature and, in the absence of suitable nourishment and at temperatures that favour the process, yeast rapidly degenerates by autolysis or consumption of its own substance.

The advantages gained by chilling the separated yeast before it is filter-pressed not only includes the retention of its full fermenting power but, at a subsequent stage, it shortens the time otherwise occupied in reducing the temperature of the pressed yeast to that of the ice house when it is placed in cold store.

From the yeast concentrate cooler the yeast flows into the

filter-press supply tank, a vessel of sufficient capacity to accommodate the yeast cream that accumulates during the interval occupied in opening, discharging, and closing the filter-press after its first filling; this tank is fitted with cooling coils connected to the yeast concentrate cooler in such a manner that the cooling medium passes through the coil before it rises in the tubes of the yeast cooler. A flanged plug cock riveted to the bottom of the tank is connected to the suction of the filter-press pump by a copper tube to which a vertical air pipe is attached. The air pipe is about $\frac{1}{2}$ inch in diameter, closed at the top with a pett cock and should be long enough to reach the level of the top of the tank.

When the filter-press is full the pipe lines and feed channels of the press are full of liquid yeast and to avoid spilling this when the press is opened air is drawn through the air pipe and pumped into the press, thus clearing the channels and displacing a considerable amount of residual moisture from the yeast.

From the yeast concentrate tank the yeast is pumped to the filter-press where it is retained as a solid block between the cloths, through which the spent wort is forced to the efflux taps and thence to the drain. Yeast is most economically filter-pressed when the speed of the press pump is adjusted to deliver a uniform flow of yeast concentrate so that very little pressure is developed while the press is filling and a uniform increase in pressure is indicated on the gauge only when the filter-press is nearly full of yeast.

This orderly routine naturally occurs at the first filling of the press, the pump is started with the separators and the yeast concentrate is pumped into the press at the same rate as it is separated. At the second filling of the press conditions are somewhat different, the yeast concentrate tank is filling rapidly with yeast cream that accumulated when the press was discharging and, with the mistaken object of saving time, the pump speed is sometimes increased to fill the empty press as rapidly as possible. In some cases no harm results but more often the yeast is forced on to the filter cloths to form a dense film that resists the passage of the spent wort; filter-pressing is then delayed and can only be completed at high pressures.

Under normal conditions it will be found that wide variations

will sometimes occur in the pressures required to obtain uniformity in the consistency in the yeast from differential brews, but the G10 yeast is invariably more difficult to press than that from the more strongly aerated G11 and G12 brews.

Filter-pressing the Yeast

A full press is indicated by an automatic reduction in the pump speed due to the back pressure from the press as indicated on the pressure gauge and a slackening in the flow of spent wort from the efflux taps on the filter plates. The same indications are sometimes given by a partly filled press of abnormal yeast and, in this case, confirmation is required before the press is opened. The time factor and the volume of yeast separated is generally sufficient indication of the progress of the work, but in the case of exceptionally heavy yields the point may be confirmed by blowing air through the press until the flow of filtrate diminishes at the efflux taps and then opening the blow-off cock fitted to the back of the follower plate; a flow of from 5 to 10 gallons indicates a full press but if the flow continues without slackening it is only partly filled, and pumping is resumed until the press is filled. The liquid yeast is then blown out of the feed channels and at the same time the air displaces a considerable volume of moisture from the cakes of yeast. When this flow almost ceases the pump is stopped and the filter-press opened.

Before the follower plate is drawn back, a trough made of stainless steel mounted on a frame fitted with truck castors is run under the filter-press to catch the yeast cake that falls as each plate is opened. Wooden scrapers are then used to free the cloths of adherent yeast particularly on the jointing faces between the plates and frames where, if pellets of yeast are allowed to remain, they may cause leaks by preventing a tight joint when the press is closed, As a rule the yeast is comparatively dry when it falls into the truck from the frames and often crumbles to a powder; in this condition it rapidly oxidizes by enzyme activity and if the finishing process is unduly delayed heat is developed and yeast degeneration occurs.

This development is avoided if the filter-press is rapidly discharged and when the trough is three parts full the powdery

yeast is sprayed with chilled water and kneaded until it is of the normal commercial consistency. At this point it may be extruded and wrapped in one pound packets or packed in the bulk containers and chilled before final packing. When the latter method is adopted it is essential to avoid air pockets in the bulk yeast. A loosely packed can rapidly stains even at the low temperature of the ice-house; and if the yeast is packed manually small portions of yeast are placed in the can at a time and well rammed with the hands to exclude air. The weight of a can of yeast is an indication of the effort expended in packing, and if a full can of yeast is found light in weight it is emptied and repacked.

The time occupied in packing, weighing and storing the yeast is largely influenced by the layout of the plant. Elaborate equipment is sometimes installed by means of which the discharged yeast falls on to a conveyor belt that delivers it to a hydraulic press that packs the yeast and discharges the weighed cans into the ice-house; but very nearly the same efficiency and speed may be obtained from the simplest plant with manual labour.

Provided that the quality of the yeast is normal the percentage yield of yeast is one of the most important items that appear on the ferment chart; therefore the weight of the individual cans and the total weight of the yeast recovered from the brew is carefully checked and recorded. It is possible, however, for yeast to be lost in separation and pressing, or for more yeast to be recovered than is reported, but these errors may be avoided and the weight of yeast produced may be estimated by a simple calculation that serves as a check upon its mechanical recovery. If the volume of the fermenting wort is known, the total weight of yeast that it contains may be determined at any time during, or after, the fermentation by comparing the difference in the specific gravity of the fermenting wort with that of the same wort deprived of yeast. Sedimentary yeast is naturally excluded from the calculation, therefore the yeast must be evenly distributed in the wort by aeration when the sample is taken.

From the last plate of the filter-press a sample of the yeast is removed, mixed with chilled water until it has the normal

consistency of commercial yeast and is subjected to the routine laboratory tests, analyses, and bakehouse trial described elsewhere. In order to obtain comparable results it is necessary to test the yeast from each brew at a definite interval after filter-pressing. The inconsistent results obtained when tests conducted with yeast twenty-four hours old are compared with the tests of freshly pressed yeast demonstrate the necessity for uniformity in the age of yeast taken for testing.

The changes that occur in the cells during this interval are indicated by the apparent increase in the nitrogen content of yeast stored in the ice-house. This difference was found when efforts were made to reduce the time occupied in the nitrogen determination. Instead of drying the yeast before digestion in the Kjeldahl method, wet yeast was used and the moisture determined in another portion of the same sample. By this method yeast fresh from the filter-press contained 7·9% nitrogen when calculated back to the dry substance, but twenty-four hours later the nitrogen content had increased to 8·1%. The increase continued for five days with a diminishing daily increment until it became constant at 8·5%.

That the change was not due to evaporation was confirmed by weighing cans of yeast for a similar period when it was found that the weight of yeast does not vary in the ice-house. The nitrogen content of yeast determined on the wet substance also remains constant during storage indicating that the apparent increase in the nitrogen content was due to the breaking down of non-nitrogenous solids in the yeast cell to liquids and the evaporation of this liquid during the moisture determination. That the results of the dough and sugar fermenting tests vary with the age of the yeast is well known. The dough test time will lengthen from fifty-five minutes when the yeast is fresh to sixty-five minutes after seven days storage and the sugar test will decline from 850 c.c. of water to 790 c.c. in the same time.

Containers for the storage of bulk yeast have been made of a variety of materials but the most satisfactory container so far devised consists of a sheet steel can about 16 inches deep and 16 inches in diameter; this is fitted with a strong steel hoop rivetted and sweated round the bottom to raise the can

off the floor. Two rigid handles facilitate handling and a loose fitting cover of sheet metal protects the yeast from contamination when the cans are racked in the ice-house. When empty the inverted cans are racked after cleaning and, before use they are scoured with hot water, steamed, and inverted to drain and cool.

The plates and frames of the discharged filter-press are drawn to the breast plate, but before they are brought in contact with each other stray pellets of yeast are scraped, or wiped, off the jointing faces and each plate pushed home with straightened press cloths to avoid the leaks that result from a folded press cloth when the filter-press is finally tightened.

The filter-press may be large enough to accommodate the total yield of yeast from a differential brew and, on the other hand, a small filter-press may be filled several times before the whole of the yeast is recovered. In either case it is generally found that there is either a little too much or not quite enough yeast concentrate to fill the last pressing. In the latter case the deficiency is easily rectified by mixing some of the yeast already pressed with a little chilled water in the yeast concentrate tank, adding a little more if necessary until the press is filled.

When it is found that a surplus of yeast concentrate remains after the last press is full, the excess may be recovered by opening the press and discharging the yeast from a sufficient number of frames to accommodate the remaining yeast cream. This will be found a time-consuming operation however as it takes the same length of time to fill five or six frames with yeast as it does to fill the whole filter-press; and while the odd plates are filling the dry yeast already in the press is increasing in temperature. This delay may be avoided by using the surplus yeast concentrate to moisten the powdery yeast as it is discharged from the press, but this expedient can only be adopted when the liquid yeast is no more than the amount required for moistening purposes.

From the foregoing it will be seen that the capacity of the filter-press may vary within quite wide limits without affecting the efficiency of the unit to any appreciable degree. If it is large enough to accommodate all the yeast from a differential

brew at one pressing it may be necessary to dissolve yeast from an earlier brew in order to fill the press if a deficiency should occur. In any case its capacity must be reduced by interposing a " dummy " plate when the yeast from a G10 seed yeast brew is pressed. A filter-press of sufficient capacity to press half the yield from a differential brew is usually installed and its dimensions may be estimated from the gross weight of the anticipated yield of yeast.

In the example under review the anticipated yield amounts to 75%, or 2,268 lb. and this plus 378 lb. of seed yeast represents a gross weight of 2,646 lb. One pound of yeast occupies a volume of 30 cubic inches in the press and the volume required by 2,646 lb. amounts to 79,380 cubic inches. The number of frames or chambers necessary will depend upon the dimensions and type of filter-press selected, but as the yeast cake is usually about 1 inch in thickness the number of frames necessary is easily calculated. When filter-pressing is finished and the yeast is packed, weighed and stored, the filter cloths are removed from the press and washed. In the meantime a suit of clean cloths is damped and the press dressed in readiness for the next day's operations. In some establishments the cloths are allowed to remain on the press and are used for several days before they are renewed. It is claimed that on a filter-press with a capacity of 1 ton, the cloths are impregnated with about 100 lb. of yeast, and this is lost every time that the suit of cloths is washed. This method appears to give satisfaction in plants where the pressing is almost continuous and no opportunity occurs for the growth of foreign organisms, but where pressing is intermittent the slight advantage gained in the yield may be offset by the grave risk of infection and of fouling the yeast with an accumulation of mould growths, that under these conditions may form beneath the filter plates and cloths.

The separators are dismantled each day when their work is finished, the discs and bowls are scoured with hot water, yeast nipples are probed and, after allowing the components to dry, the machines are assembled and lubricated in readiness for the next day's operations. Where the separators are in continuous use, time does not always permit them to be dis-

mantled for cleaning purposes and as an alternative they are scoured clean with boiling water while running at full speed until the discharge from both wort and yeast spouts runs crystal clear. This practice is quite satisfactory when separating yeast from spirit fermentations and commercial yeast from differential brews, but the more certain method of dismantling the machines must always be adopted before the separation of seed yeast.

The temperatures of the bulk yeast stored in the ice-house are observed each day by pressing a metal cased thermometer down into the centre of the yeast in one or more cans from each brew; these temperatures are recorded on a form kept for the purpose and the observed reduction in the temperature of the daily additions of fresh yeast is observed and noted. The temperature of the ice-house is maintained continuously between 36° and 40° F. by observation or by thermostatic control and the slight variations that may occur between these limits are registered on the chart of a recording thermometer. Although 40° F. is the maximum ice-house temperature the instrument is graduated from zero to about 70° F. to avoid straining its mechanism when the temperature of the ice-house rises temporarily during cleaning operations.

In order to secure uniformity in the quality of the commercial yeast proportionate amounts from several brews are sometimes mixed together in a machine resembling a dough mixer. Yeast blending, as this practice is known, is unnecessary when the yeast is produced from differential brews alone; but when spirit brews alternate with differential brews, blending is necessary owing to the different properties of yeast when cultivated in alcoholic and non-alcoholic worts.

Packing the Yeast

The equipment necessary in the packing department is so arranged, and every facility is provided, in order that the yeast may be packed and despatched without any appreciable increase in its temperature. The yeast blender is elevated to deliver blended yeast into the hopper of a pounding machine which discharges blocks of yeast weighing 1 lb. each on to a bench, where they are quickly wrapped in waxed paper, sealed,

packed in cartons containing from 1 to 4 lb. each and returned to the ice-house for chilling before despatch.

Parcels of yeast that have been extruded and wrapped in waxed paper resist the rough handling and high temperatures to which they are exposed in transport far better than those packed in the small hessian bags required by some consumers. Under the best conditions packing yeast in these bags is tedious and requires close supervision to ensure safe transport.

The hessian bags are at first damped to avoid any absorption of moisture from the yeast that they contain, which otherwise would be encased in a layer of powder yeast that rapidly deteriorates. In filling a bag it is held open by spikes set in a ring below a foot-operated plunger, the required amount of yeast is weighed and as small portions of this are placed in the bag they are pressed down by the plunger until the bag is nearly full, leaving sufficient of the bag material to be turned over and tightly sewn. The package is then placed under a lever press which excludes all air and forces the yeast under pressure into all corners of the bag. The package is then wrapped and labelled and returned to the ice-house for chilling before despatch.

Delivery arrangements vary with local conditions and the requirements of consumers. Shipping customers, biscuit manufacturers, and wholesale bakers are often supplied with yeast and yeast foods in bulk by direct delivery; but the greater part of the yeast is distributed to the smaller consumers who use from 1 to 50 lb. of yeast per day. The wrapped and labelled parcels for these customers are trucked from the ice-house direct to the despatch office and the weight of the yeast and address on the label attached to each parcel are checked off the office list at the same time as the vanman packs the parcel in his van.

The yeast is maintained at a uniform low temperature during transit by means of one of the numerous types of refrigerated vans commonly used for the delivery of perishable goods; occasionally a customer finds that he is overstocked with yeast and either rejects the delivered parcel or exchanges that for a package of stale yeast and it is these returned parcels that require special attention to avoid the possibility of one being despatched as fresh yeast in following deliveries.

Yeast Production Routine

In each factory the routine adopted for the production of

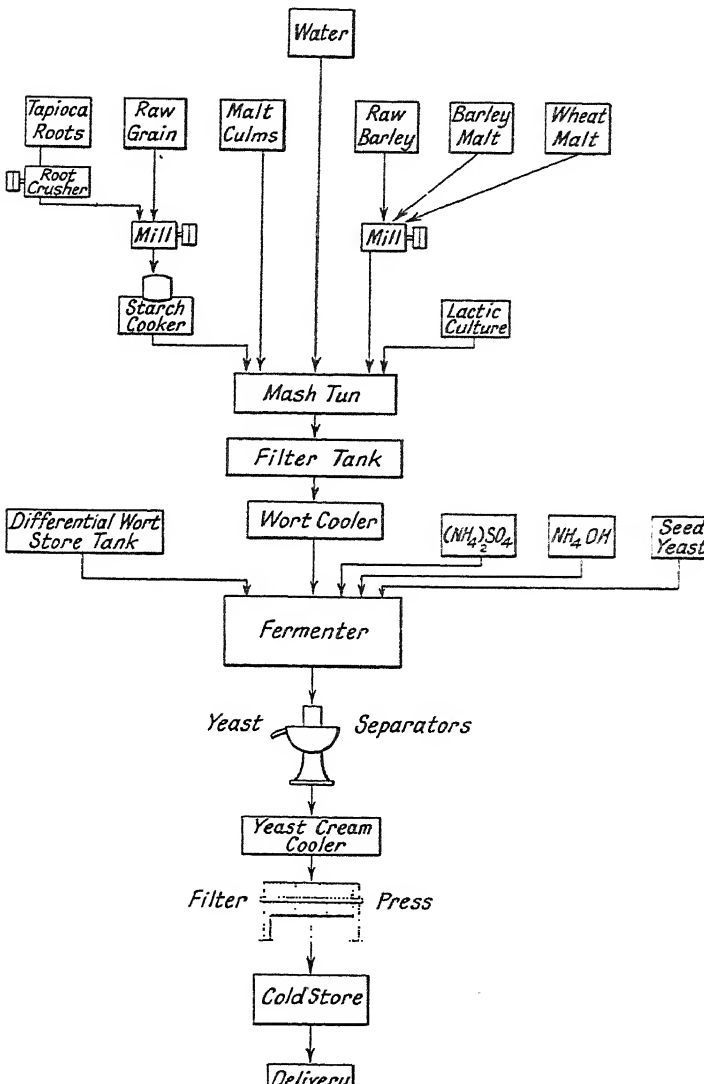


FIG. 8. Process diagram illustrating the successive operations in producing compressed yeast by the differential process from grain brews.

commercial yeast depends upon the relation of the amount of yeast sold per week to the capacity of the factory. Under normal conditions one of the most economical methods consists in producing from four to five brews per week. Continuous operation is efficient only when the output of yeast compensates for the greatly increased labour charges and cost of supervision.

Assuming that the intermittent method of production is used, and this supplies a demand for 35,000 lb. of yeast per month of four weeks, sixteen brews will be required or four brews of 1,371 kg. (27 cwts.) per week. One G10 brew will provide sufficient seed yeast for two G11 brews and a surplus of 356 lb. These quantities are calculated on the conservative estimate of a 75% yield of yeast from each G11 and G12 brew, thus producing 35,017 lb. of yeast for the month. Brewing and fermentation control are both simplified, and the weekly yields forecasted when the factory routine for the differential fermentations is arranged in a manner similar to the programme outlined below.

YEAST PRODUCTION SYNOPSIS

Date.	Day.	Mash.	Ferment.	Press.	Gross Yield. lb.	Seed Yeast. lb.	Commercial Yeast. lb. Totals.
1.	Monday	G12	—	—	—	—	—
2.	Tuesday	G12	G12	—	—	—	—
3.	Wednesday	G12	G12	G12	2,648	—	—
4.	Thursday	G10	G12	G12	2,648	—	—
5.	Friday	—	G10	G12	2,648	—	—
6.	Saturday	—	—	G10	—	997	—
7.		—	—	—	—	—	7,944
8.	Monday	G12	—	—	—	—	—
9.	Tuesday	G12	G12	—	—	—	—
10.	Wednesday	G12	G12	G12	2,648	—	—
11.	Thursday	G11	G12	G12	2,648	—	—
12.	Friday	—	G11	G12	2,648	—	—
13.	Saturday	—	—	G11	—	2,648	—
14.		—	—	—	—	—	7,944

YEAST PRODUCTION SYNOPSIS—*continued.*

Date.	Day.	Mash.	Ferment.	Press.	Gross Yield. lb.	Seed Yeast. lb.	Commercial Yeast. lb. Totals.
15.	Monday	G12	—	—	—	—	—
16.	Tuesday	G12	G12	—	—	—	—
17.	Wednesday	G12	G12	G12	2,648	—	—
18.	Thursday	G12	G12	G12	2,648	—	—
19.	Friday	—	G12	G12	2,648	—	—
20.	Saturday	—	—	G12	2,648	—	—
21.		—	—	—	—	—	10,592
22.	Monday	G12	—	—	—	—	—
23.	Tuesday	G12	G12	—	—	—	—
24.	Wednesday	G12	G12	G12	2,648	—	—
25.	Thursday	G11	G12	G12	2,648	—	—
26.	Friday	—	G11	G12	2,648	—	—
27.	Saturday	—	—	G11	—	2,648	—
28.		—	—	—	—	—	7,944

Seed Yeast surplus 593

Total Commercial Yeast 35,017 lb.

Spent Grains. The spent grains remaining in the filter tank are allowed to drain until they are in a suitable condition to handle. Draining is expedited by unscrewing the plugs below the goosenecks in front of the filter tank thus discharging the liquor that otherwise remains in the cavity between the filter plates and the bottom of the tank. The grains are discharged through a manhole in the tank bottom and gravitate through a shute into the spent grain bins, from which they are sold to stock feeders and dairymen.

As they are exceptionally rich in digestive nutrients the wet grains are in great demand as a cattle food; but as their moisture content is very high, about 80%, they cannot be kept in this condition for any length of time and, when necessary, their nutritive value and digestibility is preserved indefinitely by reducing the moisture to about 6%. They are economically

dried by first expressing the excess moisture by means of a machine in which an archimedian screw forces the grains through a finely perforated throat, tapered progressively to increase the pressure before discharge. The remaining moisture is then evaporated in a rotating dehydrator heated with the exhaust steam from the factory. The dried grains may be sold in that condition or blended with other material to produce a calf and cattle food that finds a ready sale at remunerative prices.

CHAPTER V

COMPRESSED YEAST PRODUCTION FROM MOLASSES AND INORGANIC NITROGEN

ALTHOUGH Pasteur in his memorable controversy with Liebig conclusively demonstrated that yeast could be nourished and propagated in pure cane sugar solutions with ammonia as the only source of nitrogen there was no practical application of that knowledge for over forty years later until Delbruck and Hayduck, in 1910, discovered that potato mashes could be fertilized with ammonium sulphate, the ammonia being partly converted into protein. It was found, however, that the utilization was incomplete and, in its application, the process left much to be desired from the economic standpoint.

The suggested utilization of inorganic nitrogen in the commercial production of compressed yeast was further advanced when Dr. Lange succeeded in producing yeast in large quantities from solutions of sugar and ammonium salts, and although the tests of the yeasts thus produced did not always give uniform results the process more than doubled the yields formerly obtained in yeast factories.

Beet Sugar Molasses. Dr. Hayduck then undertook the development of a process for the production of compressed yeast, without alcohol formation, from beet sugar molasses and ammonium compounds and succeeded in producing 170 kg. of yeast from 100 kg. of sugar. This result was sensational and provided a stimulus to further research in yeast production. The high yields of uniform quality yeast that are now produced are due to the development of processes that followed the successive introduction of rational methods of seed yeast propagation, the differential addition of strong wort, efficient aeration, the maintenance of the optimum *p*H in the fermenting wort and control of the concentration of nitrogen in the fermenting wort by the formalin titration.

Beet sugar molasses is the residue from which more sugar cannot be profitably extracted and contains most of the organic acids, nitrogenous and non-nitrogenous bodies and mineral constituents of the beet juice remaining after the crystallization of the sugar. Owing to its disagreeable odour and taste, beet molasses is not suitable as a food for human beings. It is utilized either by mixing with dried beet pulp and other substances for use as a cattle food; or by fermenting it for the recovery of industrial alcohol, or compressed yeast. It contains between 45 and 55% of cane sugar that is prevented from crystallizing, in time for its recovery in the sugar refinery, by the impurities present; but during its storage in the yeast factory molasses tanks an appreciable percentage of this sugar slowly separates out and is found later as a heavy deposit on the tank bottom.

Beet molasses contains only a very small amount of invert sugar and of the other optically active substances, malic, metapectic, and alkaline solutions of aspartic acid are lævorotatory besides the invert sugar and beet gum. Glutamic acid and acid solutions of aspartic acid exercise a right-handed rotation and these interfering substances, of which dextran and beet gum are the most optically active, tend in a great measure to neutralize the effect of each other.

Owing to the salts and other extractive matters having different solution densities from that of sugar the specific gravity of beet molasses does not indicate the amount of water present as it does in other sugar solutions. The technical value of molasses will be found to vary according to the methods adopted in its production and the equipment of the refinery, but on the average its most important constituents are present in the following proportions :—

Sugar . .	50·85%	Water . .	17·4%
Ash . .	9·13%	Nitrogen . .	0·54%

It readily penetrates the joints of wooden containers and for that reason it is transported either in tanks or in steel drums of about 40 gallons capacity, in which it may be stored or discharged and pumped to a bulk store tank. Bulk storage has obvious advantages including the blending of different con-

signments, and as this equalizes the specific gravity, the volume equivalent of the weight required for brewing may be calculated with accuracy; and if the storage tank commands the vessel in which the molasses is treated and clarified before fermentation, its withdrawal and measurement is simplified by gravitation.

Preparatory Treatment of Molasses

Beet molasses is a dark brown or black viscous liquid with an unpleasant odour and taste and a specific gravity ranging between 1·3 and 1·4. Before it is used for the cultivation of yeast it must be treated by a suitable process to effect the separation and elimination of substances that are not only harmful to the yeast but may completely inhibit its growth. Boiling undiluted molasses with sulphuric acid is considered sufficient preliminary treatment when the molasses is afterwards mixed with a grain brew; the cereal mash dilutes the molasses and, by retaining precipitates and absorbing colouring matter, the grain residues clarify the molasses during the souring and filtration processes.

The sulphuric acid treatment neutralizes the lime used in the manufacture of sugar, sets free the volatile acids and breaks up nitrites, thus liberating nitrogen peroxide. The solution is then heated with steam and boiled for fifteen minutes to expel the volatile acids and the oxides of nitrogen, and added to the grain mash when required.

In the preliminary treatment of molasses intended for the propagation of yeast in worts derived solely from molasses, the impurities are precipitated and removed from the diluted solution by filtration or sedimentation. The well known tricalcium phosphate precipitation process of clarifying solutions was one of the first applied to the clarification of molasses and is still extensively employed for that purpose; this treatment has no bleaching effect but it is economical and produces a brilliant wort after eight hours' sedimentation.

An alternative process, clarification by the precipitation of aluminium hydrate in the molasses solution, is more rapid but finds less favour owing to the extra plant required, it decolourizes the solution slightly and as the wort may be

immediately separated from the sediment by filtration, very little time is lost in the preparatory treatment.

In the process for the clarification of molasses by tricalcium phosphate precipitation the treatment vat is fitted with a mechanical agitator and steam coil, and charged with a volume of molasses equivalent to the weight required for the brew. In the example under review the specific gravity of the crude molasses was found to be 1·4, or 14 lb. per gallon: 3,024 lb. (1,371 kg.) thus occupies a volume of 216 gallons. Water is then added with constant agitation to ensure a uniform concentration until the density of the solution indicates between 19° and 20° Balling and its alkalinity is neutralized by the addition of sulphuric acid.

The amount of acid required to neutralize the solution varies considerably in different consignments even though the degree of alkalinity indicated is the same, but the agitation and cautious addition of sulphuric acid is continued until the solution contains 1° of acidity, determined as sulphuric acid by titrating 100 c.c. of the molasses solution against N/1 NaOH with neutal litmus paper indicator. The solution is then heated to 150° F. by the admission of live steam.

Calcium superphosphate is sold with a P_2O_5 content that varies according to the requirements of consumers, therefore an amount is weighed and added to the agitated molasses solution that corresponds to 1 lb. of finely ground superphosphate, containing 18% P_2O_5, to every 100 lb. of crude molasses used in the brew. Heating is then resumed and nitrites are dissociated by vigorously boiling the molasses solution for at least 10 minutes.

Milk of lime is prepared by first slaking quicklime in the proportion of ½ lb. to every 100 lb. of crude molasses and then adding water to produce a mixture with the consistency of thin cream. The molasses solution is agitated after standing about an hour and the lime milk is added through a coarse sieve and forms a finely divided precipitate that only slowly subsides. The alkalinity is then determined in 100 c.c. of the solution by titration with N/1 acid and, after ten minutes' stirring the agitators are stopped, the vat is closed and the solution stands perfectly motionless for at least eight hours'

sedimentation; its immobility is assured by removing the belt or locking the agitator mechanism in an immovable position.

The sediment formed during this interval consists of tri-calcium phosphate and calcium sulphate mixed with organic matter removed from the solution and is found later as a black compact layer about 1 inch deep on the bottom of the vat. The supernatant molasses solution is a brilliant rum-coloured liquid entirely free from suspended matter, and is drawn off, without disturbing the sediment, by means of two or three taps screwed into the vat staves at varying heights, the lowest being placed not less than 2 inches from the bottom of the vat. As an alternative a syphon may be used, but in this case, as the liquid is drawn off and its surface approaches the bottom of the vat, great care is necessary to avoid disturbing the sediment.

The clear solution is conveyed by means of a hose from the taps to the pump suction box, where the flow is continually examined in a strong light through a small test tube, and if any turbidity, or solid matter in suspension, is observed the flow is immediately stopped and the remainder of the solution reserved for further treatment.

The reaction of the clear solution is observed and its degree of alkalinity again determined, and if any reduction is found on comparison with the previous determination bacterial infection is indicated. The total sugar content of the solution is determined as dextrose either by titration with 10 c.c. of Fehling's solution using methylene blue internal indicator, or by Bertrand's method of sugar estimation, and expressed as a percentage of the molasses brewed. This information is required for the purpose of comparing the yield of yeast with the theoretical yield from a standard brew containing 50% of sugar.

After the solution is elevated to the wort cooler it gravitates to the wort store tank at 55° F. and remains undisturbed until required. When the flow of this solution from the treatment vat ceases, the taps are closed and the sediment is mixed with water at 180° F. until its volume equals half that of the original solution. It is then heated to boiling point and

allowed to stand motionless for six hours' sedimentation. After this interval the clear solution is drawn off, taking the same precautions to avoid disturbing the sediment, and pumped over the wort cooler from which it flows to the fermenter at 78° F.

The proportion of the molasses in solution that is retained in the wort store tank to supply the differential additions of strong wort and that used for starting wort in the fermenter is estimated in a similar manner to that described in the division of wort from grain brews. The 1,371 kg. of molasses are diluted to a volume equivalent to fourteen times the weight of the original molasses, $1{,}371 \times 14 = 19{,}194$ litres. The store wort, containing 90% of the extract, amounts to $6/14 \times 19{,}194 = 8{,}226$ litres, and is represented by the first molasses solution drawn off the sediment and pumped to the wort store tank; its further dilution is adjusted when the starting wort in the fermenter receives attention.

The fermenter is charged with $8/14 \times 19{,}194 = 10{,}968$ litres of wort containing about 10% of the extract. This wort is at present represented by the extremely dilute solution obtained by washing the molasses sediment and its density is adjusted to that required by the addition of strong wort from the store tank. The further dilution of the worts in the fermenter and store tank to their specified volumes with chilled water follows the division of the extract specified above.

The Clarification of Molasses by the Precipitation of Aluminium Hydrate: In this process the treatment vat is charged with the same quantity of molasses (216 gallons) as in the previous example, it is diluted with its own volume of water and thoroughly mixed by agitation. A 10% solution of aluminium sulphate is prepared by dissolving the salt, in the proportion of 1·5 lb. to every 100 lb. of molasses, in hot water and diluting with water to the required volume. After the addition of this solution to the molasses the mixture is heated with live steam and boiled for sixty minutes. The molasses is then further diluted and its temperature reduced by the addition of an equal volume of cold water.

Aluminium hydrate is precipitated in the molasses solution by the addition of a 10% lime milk, prepared by slaking a quantity of quick lime equivalent to 2·5 lb. of lime to each

100 lb. of molasses, and diluting it to the required volume. After the addition of the lime milk the molasses solution is heated and boiled gently for two hours. The steam valve is then closed and the solution allowed to remain motionless for fifteen minutes. A thick scum rises to the surface during this interval and clarification is assisted if this scum is completely removed by skimming.

Although the precipitate is too light and bulky to be separated by sedimentation it is in a condition that permits a rapid filtration and produces a brilliant filtrate which, after cooling and dilution, is ready for immediate fermentation. A small filter-press is steam sterilized with formalin and dressed with clean sheets through which the molasses solution is filtered either by gravitation or a force pump, according to the disposition of the plant. Infection may be avoided by pumping the hot filtrate direct to the wort store tank but unless the cooling system is highly efficient it will be found that fermentation temperatures are controlled with less difficulty if the filtrate is chilled to a temperature not exceeding 60° F. before it is diverted from the wort cooler to the wort store tank.

The start wort is prepared by discharging a volume of the filtrate, that represents in all a total of 10% of the extract, from the store tank into the fermenter, where it is diluted with water to produce 10,968 litres at 78·5° F. The volume of the strong wort is adjusted to 8,226 litres by diluting the filtrate remaining in the store tank with water chilled to 60° F.

Chemicals Required in Molasses Worts

Clarification by the tri-calcium phosphate precipitation process entirely removes phosphates from the molasses solution and, as yeast can neither ferment or propagate in the absence of phosphates, the deficiency is rectified by the addition of phosphates in solution at intervals during fermentation. The total amount required may be determined by analysis of the yeast, but a necessary surplus is provided by using 5 lb. of superphosphate (18% P_2O_5) per 100 lb. of crude molasses.

The soluble phosphates, $CaH_4(PO_4)_2$, are extracted by adding the superphosphate to water, acidified with 0·5% sulphuric

acid, in the proportion of 2 lb. of superphosphate per gallon of water. After stirring the mixture for ten minutes the sediment is allowed to settle and the clear supernatant liquid is syphoned into a separate vessel. The sediment is then washed by agitation with acidulated water made up to the original volume in the vessel and the clear solution is syphoned and mixed with the first solution in a graduated wooden container. One-tenth of the total volume is added to the start wort in the fermenter before it is inoculated with seed yeast and the remainder is divided and added to the fermenting wort each hour in quantities that are proportional to the volumes of the differential flow of strong wort.

Although the assimilable nitrogen content of molasses wort is so small that it is ignored in estimating the total amount of nitrogen required for the brew, the wort is so heavily charged with protein and other buffer substances that it is necessary to maintain the hydrogen ion concentration as near *p*H 4·5 as possible during the fermentation. Very little, if any, aqueous ammonia will be required owing to the alkalinity of the store wort but at certain periods of the fermentation and particularly during the two hours' flow of strong alkaline wort after the last addition of $(NH_4)_2SO_4$ appreciable amounts of sulphuric acid may be necessary to preserve the optimum acidities in the fermenting wort.

Ferment Chart

DATE..... BREW No..... GENERATION 12. FERMENTED by.....

RAW MATERIAL.		CHEMICALS.		MASHING AND SOURING.					FILTRATION.
	Kilo-grams.		Kilo-grams.	Time.	Mash Acidity.	Gravity Balling.	Temp. deg. Fahr.	Mash heated to	
Maize .	—	—	—	1 p.m.	7 kgs. of Lime slacked.				
Wheat .	—	Lime	7	8.30 a.m.	phosphate solution prepared.				Time started.
Barley .	—	$Al_2(SO_4)_3$	—	10 a.m.	,,	,,	syphoned.		Time finished.
Tapioca .	—	$(NH_4)_2SO_4$	90	12 noon	,,	,,	syphoned.		Hours.
Molasses .	1,371	NH_4OH	—	5 p.m.	Molasses cleared				Gravity.
Wheat Malt	—	H_2SO_4	10	4 a.m.	,,	syphoned.			Mashed.
Chev. Malt	—	$CaH_4(PO_4)_2$	84	11 a.m.	,,	syphoned.			
Cape Malt	—	[272 litres of Phosphate solution = 12·6 kgs. P_2O_5 (18% of 70 kgs.)]							
Culms .									
Total .	1,371								

Fermentation Record

Time in Hours.	Temp. deg. Fahr.	Gravity deg. Balling	Acid degrees	*p*H	Formalin Number	Cubic Feet of Air per Minute.	Litres of Strong Wort added	Litres of NH_3	Kilograms of $(NH_4)_2SO_4$	Kilograms of H_2SO_4	Litres of $CaH_4(PO_4)$ solution.
1 p.m.	78·5	0·75	0·3	4·4	0·05	498	—	—	10·0	2·0	27·25
2 ,,	78·5	1·2	0·3	4·5	0·9	806	Start.	—	—	—	10·75
3 ,,	78·5	1·4	0·4	4·5	0·6	806	411	—	—	—	12·0
4 ,,	78·5	1·5	0·4	4·6	0·3	806	452	—	7·5	—	13·0
5 ,,	78·5	1·65	0·6	4·5	0·35	806	494	—	7·5	—	14·0
6 ,,	78·5	1·8	0·7	4·6	0·3	1,048	617	—	7·5	—	15·0
7 ,,	78·5	2·0	0·9	4·7	0·3	1,048	740	—	10·0	0·5	18·0
8 ,,	78·5	2·2	0·9	4·4	0·4	1,048	864	—	12·5	—	23·0
9 ,,	80·0	2·5	1·0	4·6	0·4	1,048	987	—	12·5	0·5	26·0
10 ,,	81·5	2·7	1·1	4·3	0·35	1,048	1,070	—	12·5	—	30·0
11 ,,	83·5	2·9	1·2	4·5	0·3	1,048	1,070	—	10·0	1·0	33·0
12 ,,	85·0	3·0	1·4	4·6	0·25	1,048	1,110	—	—	1·0	30·0
1 a.m.	86·0	3·0	1·6	4·5	0·2	498	411	—	—	0·5	20·0
2 ,,	86·0	3·0	1·7	4·3	0·15	Feeble.					
3 ,,	Separated Wort	1·3						Totals	90 kgs.		272 litres.

Extract, Yields and Notes

Strong wort 8,226 litres, 10·5 gravity Balling = 863·73 Balling hectolitres.
Start wort 10,968 litres, 0·75 gravity Balling = 82·26 Balling hectolitres.

Sugar 48·5%. Total = 945·99 Balling hectolitres = 69·0% extract.

Yield of yeast.
Kgs. of yeast gross, 1,400.
Kgs. of seed yeast, 275. Brew No. Gen.
Kgs. of yeast nett 1,125 = 82·0%.

Dough test, 54 minutes.
Sugar test, 840 c.c.
Protein, 52·0%.

Calculated yield of yeast.
Wort + yeast, 3·0 deg. Balling = 1·0120 specific gravity.
Wort − yeast, 1·3 deg. Balling = 1·0052 specific gravity.
Difference in specific gravity = 68 = kilograms of yeast, 1,422 gross.

Preparatory Treatment of Materials

The following is a summary of the treatment of materials in preparation for the fermentation of a molasses brew :—

First Day. Slake 7 kg. of quick lime and prepare a 10% milk of lime.

Charge the treatment vat with 1,371 kilograms of molasses (27 cwt.) and dilute with hot water to 20° Balling. Acidify to 1° of acidity with sulphuric acid. Add 14 kg. of finely powdered $CaH_4(PO_4)_2$. Boil the solution ten minutes and allow it to stand one hour. Add the milk of lime, stir ten minutes, close the vat and lock the agitator for eight hours.

Prepare a phosphate solution by mixing 70 kg. of $CaH_4(PO_4)_2$ in 340 litres of water and 1·7 kg. of sulphuric acid. Syphon

the clear solution into another vessel. Wash the sediment with acidified water, syphon and mix the two solutions in a graduated vessel and report the total volume.

Second Day. Decant the clear molasses solution from the treatment vat and pump it to the wort store tank at 55° F. Wash the sediment by half filling the vat with water at 185° F. Stir and lock the agitator for six hours. Decant the clear solution and pump it to the fermenter at about 85° F.

Dissolve and filter 100 kg. of $(NH_4)_2SO_4$. Charge the fermenter with 10,968 litres of 0·75° Balling wort at 78·5° F. and 0·3° acidity. Dilute the store wort to 8,226 litres with chilled water and supply 20 kg. of sulphuric acid and 275 kilos of seed yeast.

The Differential Fermentation of Molasses Worts

Before commencing the fermentation the results of the routine tests of the start wort are recorded on a ferment chart similar to the copy reproduced on p. 133. An examination of these commencing figures shows that the very low concentration of assimilable nitrogen indicated by the formalin number 0·5 was corrected by the addition of 10 kg. of $(NH_4)_2SO_4$ and this is reflected in the higher value shown by this titration after sixty minutes' fermentation.

It is not practicable to determine the phosphate content of the wort in time for the determination to be of any service in controlling the concentration of this constituent and experience demonstrates that analysis is unnecessary if the quantity of superphosphate specified is treated and applied as directed. In this example 272 litres of solution were obtained by leaching the superphosphate and of this volume 27·25 litres, approximately 10% is applied as shown on the ferment chart in fractions that increase with the increased volumes of strong wort.

An appreciable increase in the amount of seed yeast is necessary for the fermentation of molasses worts than that required for grain brews; the quality of the yeast produced does not suffer if a smaller amount of seed yeast is used, but the maximum yields are not obtained unless the fermentation is commenced with seed yeast amounting to 20% of the weight of molasses brewed.

The same volumes of air are supplied to a molasses brew as to a grain brew, unless the percentage of sugar in the crude molasses exceeds 50%. When yeast is provided with sufficient oxygen for its needs, fermentation is suppressed by the reproductive activity of the yeast and the formation of alcohol ceases. Alcohol can only be produced in a differential brew when aeration fails or by the fermentation of molasses exceptionally rich in sugar with deficient aeration, and, as yeast increase and alcohol are mutually exclusive in production, the formation of 1 litre of alcohol reduces the yield of yeast by over 3 kg.

During the first seven hours of the fermentation the temperature is maintained at 78·5° F., and is then allowed to rise at a uniform rate until the maximum, 86° F., is reached during the last hour of the differential flow of strong wort. The rate of yeast increase is always greater at low temperatures, and, within the limits that can be taken into consideration, alcohol production is stimulated by high temperatures, but a distinguishing feature of this process is that the factors which accelerate yeast cell multiplication are in equilibrium, not only at low temperatures for more than half the time, but also during the later period when the increasing volume of the fermenting wort allows higher temperatures to stimulate the secretion of alcoholase in the cells without alcohol production.

After the addition of seed yeast the control of the fermentation follows the same lines as detailed in describing the differential fermentation of an all-grain brew, except that slight variations are imposed by the different nature of the molasses wort involving the periodical addition of phosphates in solution, the maintenance of a higher numerical *p*H and the addition of appreciable volumes of free H_2SO_4 to neutralize the effect of the flow of alkaline strong wort. In molasses brews froth formation is generally slight, and on the rare occasions when large volumes are formed it is readily dispelled by the addition of a small amount of melted fat.

The volumes of air, strong wort and phosphate solution periodically supplied to the brew are entered on the ferment chart before fermentation commences, and the course of the fermentation is clearly shown by the recorded details. The

progressive increase in the gravity Balling during fermentation is an indication of the rate of cell increase, and the amount of yeast in the brew may be determined at any period of the fermentation by first multiplying the weight of the fermenting wort by the specific gravity of yeast (1,090), and then by the difference in the specific gravity of the wort with yeast and wort without yeast (see also p. 167). For the purpose of this estimation the specific gravities of the wort may be determined with sufficient accuracy by the conversion of the indications of the Balling saccharometer (4 × degrees Balling + 1,000 = specific gravity).

As soon as the routine tests are complete at the end of the fermentation separation commences, but before the yeast concentrate is chilled or filter pressed it is subjected to a washing process in order to avoid the production of tinted yeast. The dark colour of the yeast concentrate is due not only to the wort in which the yeast is suspended, but to solid colouring matter adhering to the cells, and on which it appears to become fixed if the yeast is pressed in this condition.

This development may be avoided by bleaching the molasses solution before fermentation, but this cannot be accomplished without further cost during its preliminary treatment. As an alternative the yeast is restored to its normal colour by washing the concentrate before it is pressed. As the yeast flows from the separators it is discharged into a reservoir of sufficient capacity to contain all the concentrate from the brew (about 3,850 litres), and as soon as the fermenter is emptied it is refilled to the original volume of the brew with cold water and yeast concentrate, sulphuric acid is added to increase the acidity of the solution to *p*H 3·0, the mixture is then strongly aerated for thirty minutes by injecting the maximum capacity of the air compressor. After this interval separation recommences and filter-pressing follows the normal routine.

Seed Yeast Production from Molasses Worts

The successful production of commercial yeast from molasses and inorganic nitrogen in non-alcoholic fermentations effected a considerable reduction in the factory equipment

required for the economical disposition of the immense volumes of alcoholic wash that were, until then, inseparable as a by-product of compressed yeast manufacture and, in addition, it released most of the space required for the storage of grain and other brewing materials; only a small portion of this space being retained to store the grain necessary for seed yeast production, while investigations were directed to the perfection of methods for the cultivation of seed yeast in molasses worts, and eventually the most efficient process finally evolved follows the procedure described in the production of seed yeast from grain worts.

It is possible to isolate and propagate yeast pure cultures in molasses wort with inorganic nitrogen, but a far superior commercial yeast is produced from laboratory cultures grown in molasses wort with organic nitrogen. In the factory cultures the organic nitrogen progressively diminishes in amount and is replaced by $(NH_4)_2SO_4$, or ammonium phosphate, in three generations, thus avoiding an abrupt change from one form of nitrogenous nutriment to another and gradually cultivating a tolerance to inorganic nitrogen, with the result that after washing the G10 yeast cannot be distinguished from that generation grown in worts derived from grain.

The nutrient solutions for these laboratory cultures are produced by mashing molasses with 10% of its weight of malt culms. The molasses is diluted with water to 18° Balling, clarified, and acidified to 0·25° acidity with sulphuric acid and heated to 140° F. The malt culms are then added and, after mashing for three hours at 144° F. with occasional stirring, the wort is separated from the culms by filtration through a ribbed filter paper and diluted to 12° Balling by washing the culms with water at 160° F. The filtrate is then heated to boiling, refiltered, filled into flasks and fractionally sterilized by boiling for fifteen minutes on three successive days. The isolation of pure cultures and their propagation as laboratory cultures in this wort follows the routine previously outlined in the production of seed yeast.

In grain worts brewed for falling curve fermentations the ratio of assimilable nitrogen to sugar is approximately 1 to 25 and the same ratio is preserved in preparing the molasses worts

for factory cultures, but in the G6 culture wort, one-third of the assimilable nitrogen is supplied as $(NH_4)_2SO_4$; in the G7 culture the proportion of this salt is increased to two-thirds, and for the G8 and following cultures inorganic nitrogen only is supplied in the proportion of 300 gm. of $(NH_4)_2SO_4$ to each 100 litres of 12° Balling wort. The organic nitrogen is prepared in solution by mashing malt culms with three times its weight of water at 144° F. for twenty-four hours. The extract is filtered from the culms, boiled, refiltered into suitable containers and fractionally sterilized after determining the assimilable nitrogen content by the formalin titration, 1 c.c. of N/1 NaOH = 14 mg. of nitrogen per 100 c.c. of wort.

Whenever it is convenient to do so the molasses solutions required for the factory cultures are clarified together with the crude molasses issued for the differential fermentation, but if this method is not practicable the wort may be prepared by clarifying the small volumes of solution as required, adding phosphates in solution, as ammonium phosphate, or superphosphate in the proportion of 200 gm. per 100 litres of 12° Balling wort and adjusting the hydrogen ion concentration to *p*H 4·5 with sulphuric acid immediately before inoculation with the preceding culture.

The small volumes of wort used in G6 and G7 cultures may be sterilized and safely kept for several days in sealed containers, but owing to their greater bulk this method cannot be applied to the wort prepared for the G8 and G9 cultures, which may be clarified in the one operation but, after dilution to 12° Balling, it is divided into the volumes required for each vessel in which the generations are fermented; the procedure being timed in order that the G8 fraction of the wort may be prepared for fermentation and the temperature adjusted to 72° F. at the same time that the G7 culture is ready for transfer. In the meantime the 3,072 litres of this wort reserved for the G9 fermentation are chilled to 60° F. to protect it from infection in the closed G9 fermenter, where it remains undisturbed until required.

Owing to the vitality and amount of yeast produced in each generation by this process the G8 culture is completely fer-

mented in fifteen hours, and continuity of cell reproduction is assured by its immediate transfer to fresh wort.

The temperature of the wort in the G9 tank is increased to 72° F. by the admission of live steam and it is prepared for fermentation by adding sufficient H_2SO_4 to neutralize the alkalinity and increase the hydrogen ion concentration to *p*H 4·5. This is followed by the addition of inorganic nitrogen and phosphate, as ammonium phosphate or equivalent amounts of $(NH_4)_2SO_4$ and $CaH_4(PO_4)_2$, and its fermentation commences with the transfer of the G8 culture.

The temperature is kept constant at 77° F. for two hours and is then allowed to rise 1° F. per hour until it reaches the maximum at 84° F. seven hours later. The brew is aerated by injecting 300 cubic feet of air per minute from the beginning to end of the fermentation, and both phosphates and nitrogen are added at the start and at intervals of two hours in amounts that when totalled are calculated to provide a slight excess above that required for the production of a nett yield of 20% yeast. When fermentation is complete the lag phase, or maturing period, is reduced to thirty minutes and, after this interval, the contents of the tank are discharged into the big fermenter to commence the G10 fermentation.

The G10 Fermentation. When four brews per week are required to supply the demand for commercial yeast, it is advantageous to ferment the G10 brew at the end of the week, as this procedure not only allows the wort necessary for the preceding cultures to be prepared with that required for the differential brews, but the supervision and transfer of the cultures is more efficient and economical. In a G10 grain brew the strong starting wort becomes rapidly attenuated by dilution with the G9 culture and with the weak wort filtered from the grains during the first four hours of the fermentation, and the same result is effected in the G10 molasses brew by suitably diluting a portion of the strong molasses wort reserved for the purpose.

The same weight of molasses is required for a G10 brew as that treated for a differential brew, and after clarification the solution is conveyed from the treatment vat to the wort store tank, where it is diluted to 15° Balling with the weaker solution

Ferment Chart

DATE..... BREW No..... GENERATION 10 FERMENTED BY.....

RAW MATERIAL.		CHEMICALS.		MASHING AND SOURING.					FILTRATION
	Kilo-grams		Kilo-grams	Time.	Degrees Acidity.	Degrees Gravity Balling	Tempera-ture deg. Fahr.	Heated to.	Time.
Maize .	—	—	—	—	7 kgs. Lime slaked.				Started.
Wheat .	—	Lime	7	8.30 a.m.	Phosphate Solution prepared.				Mashed.
Barley .	—	$Al_2(SO_4)_3$	—	10 a.m.	,,	,,	syphoned.		Finished.
Tapioca .	—	$(NH_4)_2SO_4$	34·5	12 noon	,,	,,	syphoned.		Hours.
Molasses .	1,371	NH_3	—	4 p.m.	Molasses cleared.		—		
Wheat Malt	—	H_2SO_4	6·5	4 a.m.	,,	syphoned	Volume 15° B.		
Chev. Malt	—	$CaH_4(PO_4)_2$	55	10 a.m.	,,	syphoned	7,020 litres.		
Cape Malt									
Culms .									
Total .	1.371			41 kgs. superphosphate = 134 litres = 7·4 kgs. P_2O_5 (at 18%).					

Fermentation Record

Time in hours.	Temp. Fahr.	Gravity deg. Balling.	Degrees Acidity	pH	Formalin Number	Cubic Feet of Air per Minute.	Litres of Strong Wort added	Litres of NH_3.	Kilograms of $(NH_4)_2SO_4$	Kilograms of N_2SO_4	Litres of $CaH_4(PO_4)_2$ solution.
1 p.m.	72	15·0	—	—	—	—	—	—	34·5	—	134
2 ,,	72	10·3	0·5	4·3	3·6	565	Start.	—	—	—	G9 added.
3 ,,	72	7·6	0·6	4·2	—	565	4,389				
4 ,,	72·5	6·3	0·7	4·2	2·4	565	3,290				
5 ,,	72·5	5·8	0·8	4·3	—	685	2,193				
6 ,,	75·0	5·5	0·8	4·4	1·2	685	1,096				
7 ,,	77·0	4·9	0·9	4·6	—	807					
8 ,,	80·0	4·2	1·0	4·6	0·6	807	—	—	—	0·5	
9 ,,	82·5	3·4	1·1	4·6	—	807					
10 ,,	84·0	2·8	1·2	4·5	0·3	807					
11 ,,	84·0	2·3	1·2	4·5	—	807					
12 ,,	84·0	2·0	1·4	4·4	0·2	565					
1 a.m.	84·0	2·0	1·4	4·2	—	565					
Separated wort	84·0	1·35	—	—	0·18						

Extract, Yields and Notes

Strong wort . .	5,484 litres, 15·0 gravity Balling = 722 Balling hectolitres.	
Store wort . .	10,968 litres, 2·1 gravity Balling = 220 Balling hectolitres.	
Total . .	16,452 (plus G9 = 20,548 litres)	942 = 68·7% extract.

Total raw material, G10 + G9 = 1,712 kgs.
Yield of yeast, 556 kgs. = 32·4%.
Yield of alcohol, 1·33% = 218·8 kgs. = 15·9% on raw material.

Dough test, 58 minutes.
Sugar test, 860 c.c.
Protein, 51·4%.

Calculated yield of yeast.

Wort plus yeast .	2·0 deg. Balling = 1·0080 specific gravity.	
Wort minus yeast	1·35 deg. Balling = 1·0054 specific gravity.	
Difference		26 = 582 kgs. of yeast.

obtained by washing the molasses sediment, but before its division into strong starting wort and weak store wort it is mixed by aeration with the total amount of chemicals required during the fermentation. The alkalinity of the solution is neutralized and acidified to *p*H 4·5 by the addition of between 0·3 and 0·5 litre of sulphuric acid per 100 kg. of molasses; this is followed by the phosphate solution obtained from the extraction of 41 kg. of superphosphate (3 kg. per 100 kg. molasses) and about 34·5 kg. of ammonium sulphate (2·5 kg. per 100 kg. of molasses).

In the example brew the dilution of 1,371 kg. of molasses to 15° Balling produced 1,720 litres of wort. The fermenter is charged with a volume of this wort (5,484 litres) that represents between 75% and 80% of the total extract, and when this is seeded with the attenuated G9 culture the fermentation commences in wort at about 10° Balling. The 1,536 litres of strong wort remaining in the store tank is then diluted with sterile water (9,432 litres) until the original 1,371 kg. of molasses is diluted to twelve times its weight, excluding G9 culture.

5,484 litres of starting wort at 15° Balling,
1,536 litres of store wort at 15° Balling,
9,432 litres of sterile water,

16,452 = 12 × 1,371 (kilograms of molasses).

During the first four hours of the fermentation the diluted store wort is allowed to flow into the fermenter at a rate adjusted to deliver 0·4 of its total volume during the first hour, and after this interval the flow is reduced each hour to deliver a volume diminished by 0·1 of the total until the supply is exhausted, as shown on the fermentation record. In order to supply the higher percentage of seed yeast required for each of the two succeeding G11 brews, more yeast must be produced from a G10 molasses brew than from a grain brew of the same generation, and yeast cell multiplication is further stimulated by slightly increasing the volumes of air injected into the brew at temperatures and times shown in the following table :—

Time.	Cubic metres of Air per hour per kilogram of Raw Material.	Cubic feet of Air per minute required for the Brew.	Temperatures.
When G9 culture is added . .	0·70	565	1 hour at 72° F.
When wort ferments to 6·5° Ball. .	0·85	685	2 „ „ 72·5° F.
When wort ferments to 4·5° Ball. .	1·00	807	1 „ „ 75° F.
When gravity is constant for 60 minutes . .	0·70	565	then rising to 84° F. in four hours.

The progress of the fermentation is followed by recording the details of the routine tests and observations. The addition of inorganic nitrogen is seldom required, but a small amount of sulphuric acid may be necessary to correct *p*H variations. At the end of the fermentation the yeast is allowed to mature for one hour before separation commences, and it may be bleached by washing the yeast concentrate as described in the production of commercial yeast from molasses, but this procedure is considered unnecessary with the product of a G10 brew, and, as a rule, the yeast cream is chilled and filter-pressed and the yeast stored in the icehouse without further treatment.

The Mechanical Clarification of Molasses. Until recently the preparation of molasses worts, suitable for the production of compressed yeast, could only be effected by chemical treatment of the crude molasses and subsequent clarification of the solution by the tedious sedimentation process described in the preceding pages, but after considerable research a mechanical molasses clarifying and processing installation was perfected by Ramesohl and Schmidt, and its introduction to the industry completely revolutionized this feature of brewing operations.

The economy in space effected by this refinement is considerable, and the complete plant has reached such a high order of efficiency as to be regarded as indispensable in the treatment

of molasses as yeast separators are in the recovery of yeast from fermented wort.

By means of this installation crude molasses is diluted to any specific gravity and rate of flow that may be necessary to suit the conditions required in various processes of yeast culture. With the separator inlet and discharge closed against atmospheric contamination, the solution is sterilized and clarified under pressure, and the separator, thus arranged, can also function as a pump to deliver measured volumes of froth-free sterile wort at any point desired in the factory.

The Continuous Process

The continuous production of yeast from one brew is effected by the continued addition of strong wort to an extended differential fermentation and the simultaneous withdrawal of an equivalent volume of fermented wort in order to preserve a uniform volume of wort in the fermenter. The limitless extension of the continuous process is prevented by the rapid growth of aerobic micro-organisms in the fermenting wort, and, although considerable effort has been directed to avoid or reduce this infection, the fermentation is rarely extended beyond twenty-four hours with any degree of confidence.

In order to reduce infection risks the interior walls of the building are finished with a hard smooth and impervious surface ; wood or any other substance of a porous or absorbent nature is not permitted inside the building. The plant, fittings and utensils are designed to facilitate cleansing and are so disposed that the building may be sealed and steam sterilized with formalin before each fermentation. Infection may then be found in the most unexpected places ; the seed yeast is one of the most fertile sources, and this condition is due to its contamination during separation, filter-pressing and storage. A faulty air filter or the infection distributed by soiled clothing may often cause the early termination of what was intended to be a continuous fermentation.

When compared with the yields obtained by the intermittent method, the amount of yeast produced by the continuous process is one of its most attractive features. From the comparatively small plant required to produce 1,028 kg. of

yeast, 75% yield, from 1,371 kg. of raw material by the former method, three times this amount, 3,084 kg. at the same percentage yield can be produced if the absence of infection permits the extension of the differential additions of strong wort to occupy a total fermentation period of twenty-four hours.

The process follows the normal routine of a differential brew until the volume of wort in the fermenter is 3 metres deep. At this point the flow of strong wort is adjusted so that a constant volume is delivered each hour until fermentation ceases. An overflow pipe set at 3 metres from the tank bottom automatically discharges an equal volume of the fermenting wort into small tanks, where the yeast is matured before its separation.

An adequate supply of strong wort is provided by three grain mashes of 1,371 kg. each, or the equivalent in other material. Mashes are prepared at intervals of eight hours, and, where two tuns are available, mash saccharification and acidification follows the normal routine, but if one mash tun only is used, the souring periods of the supplementary mashes are curtailed or omitted. Sterilization then follows saccharification and the filtered wort is pumped to the store tank with as little exposure to atmospheric contamination as possible. Under these circumstances variation in the concentration of the store wort is unavoidable, but its gravity Balling may be kept within reasonable limits if wort above 10° Balling only is pumped to the wort store tank, and, after the first mash, the weaker filtrates are reserved in a weak liquor vat for use as first sparge on the grains of succeeding mashes.

The preliminary fermentation commences with the addition of from 12·5% to 20% of seed yeast according to the percentage of molasses brewed to the normal volume of starting wort; the routine procedure of a differential brew is then followed until the fermenting wort is 3 metres deep and the continuous phase of the process commences. The blower speed is not reduced at the end of this preliminary fermentation, but the maximum volume then injected is continued for the full period of active fermentation and until one hour after the flow

of strong wort ceases; the air volume is then reduced to an amount sufficient to keep the yeast in suspension until it is separated from the spent wort.

During the continuous phase the differential strong wort flows into the fermenter at a uniform rate to deliver, in this example, 1,110 litres per hour; and, although the volume actually delivered each hour may alter slightly when the wort level rises in the store tank with fresh supplies of wort from the supplementary mashes, the order of accuracy is such that once the wort delivery cock is adjusted further attention to this detail is rarely necessary. As soon as the fermenting wort reaches the overflow tube from the fermenter to the first maturing tank the latter vessel fills with fermenting wort at the same rate that the strong wort flows into the fermenter, and to ensure the consumption of any residual nutriment the wort is aerated by adjusting the air flow to deliver the same volume of air per minute as there is wort in the tank.

The capacity of each maturing tank is equal to one hour's differential flow, and the yeast suspended in the fermented wort is matured for two hours in flowing through these vessels before it is separated. The maturing tanks, which may be separate vessels or one larger tank divided into two equal compartments, are constructed of sheet steel and fitted with air-distributing tubes and gate valves, flushing cocks and an overflow aperture so placed that when full the first tank discharges into the second, the wort in this tank is aerated at half the rate of that in the first tank and when full overflows continuously into the separator feed main.

One of the smallest yeast separators has double the capacity required to separate the 1,110 litres of fermented wort that overflows each hour during the continuous phase of the fermentation, and, as a rule, this type of separator is inefficient unless it is supplied with the full volume of wort specified by the makers. The deficiency is rectified by supplementing the fermented wort with a continuous stream of cold service water. When fermentation ceases, all the available separators are pressed into service for the purpose of separating, chilling and pressing the yeast from the main fermentation as rapidly as possible.

Molasses and Grain Brews

Although no difference can be detected between the yeast produced from molasses and that produced from grain, either by analyses or practical application, it is considered in some quarters that a better product is obtained from a mixture of molasses and grain than from an all-molasses brew or an all-grain brew. This may be due to differences in factory equipment or technique but in any case a reduction in cost follows the use of molasses as a constituent of grain brews and in addition, a larger proportion of malt culms may be incorporated in the mash, the mashing process is simplified and the slight bleaching effect of the grain residues partly decolourises the wort and produces yeast of the normal creamy tint without its further treatment. The proportion of molasses that may be used is limited only by the necessity to mash enough grain to form a filter bed with sufficient depth and texture to retain the finely divided solid matter suspended in the mash liquor and thus produce a brilliant filtrate.

In addition to the methods of clarifying molasses previously described the cheaper "Lime process," may be employed when molasses is prepared for inclusion in grain brews. The molasses is diluted to between 18° and 20° Balling with warm water, and while the solution is agitated by aeration, or a mechanical agitator, slaked lime in an amount equal to 1% of the weight of crude molasses is added through a coarse sieve and the mixture boiled vigorously for ten minutes. The precipitate formed by this treatment removes some of the colouring matter from the solution and, after seven hours sedimentation, forms a dense black deposit on the bottom of the vat. The clear solution is then decanted, or syphoned, off the sediment into a separate vessel, acidified to 0·25° acidity, and added to the mash tun after the grain mash is saccharified but before it is inoculated with the culture of lactic acid bacteria.

The installation of a molasses treatment vat is essential when any one of the precipitation processes is employed to clarify the molasses but where the extra plant is not desired satisfactory results may be obtained by treating the crude molasses with sulphuric acid in the starch cooker after the

gelatinized starch is discharged from that vessel in the mashing process. The undiluted molasses is acidified to 1° acidity, boiled vigorously for thirty minutes and diluted to 20° Balling by the addition of cold water and then added to the grain mash without further treatment. As an alternative the molasses may be diluted and then acidified to 1° acidity before boiling, but in this case the acidity is reduced to about 0·15° by the addition of lime after boiling and the temperature is reduced to 150° F. by means of the cooling coil before its addition to the mash tun. The example mash is composed of one-third molasses and two thirds grain and culms in the following proportions.

Molasses .	457 kg.	=	1005·4 lb	= 33·3%
Raw grain .	480 ,,	=	1056·0 ,,	= 35·0%
Malted grain	160 ,,	=	352·0 ,,	= 11·6%
Malt culms .	274 ,,	=	602·8 ,,	= 20·0%
Totals	1371 kilos.		3016·0 lb.	

Diastase is most active in the mash at *p*H 5·0 and starch conversion is retarded and finally ceases the more that *p*H diverges from this point. The actual acidity of a mash prepared under normal conditions from sound materials is invariably about *p*H 5·0 and no adjustment is necessary to obtain a complete conversion of the starches to sugar, but the addition of molasses disturbs this natural balance and it is thus necessary to saccharify the grain starches before the addition of the molasses solution to the mash.

In preparing the mash the raw grain is gelatinized in the starch cooker with 3·5 times its weight of water and allowed to stand until required. In the meantime the malt is peptonized in the mash tun at 112° F. with three times its weight of water for sixty minutes. After this interval the gelatinized material is added to the malt and saccharified at 145° F. for two hours. The fluidity of the mash is influenced, at this point, by the type of raw grain employed and if it is considered then that the full quota of malt culms cannot be absorbed, a portion is reserved for inclusion later with the molasses solution.

The completed mash is soured at 132° F. for the first five hours and after increasing the temperature to 138° F. souring continues for a further eight to ten hours with five minutes' agitation at intervals of four hours to equalize the mash temperature. The sterilization of the mash and the filtration and division of the extract into strong wort and starting wort follows the normal routine, and fermentation commences with the addition of ammonium sulphate, 15% of seed yeast, and the injection of the same volumes of air required in the differential fermentation of an all grain brew.

The total amount of nitrogen required during fermentation is governed by the percentage of molasses used in brewing, but the addition of ammonia and ammonium sulphate is proportioned to maintain a uniform *p*H 3·5 during the main fermentation period. All other factors that influence the fermentation are controlled according to the principles of the differential process.

Yeast from Starch Sugar Brews

The sugar formed by the acid hydrolysis of starch and variously known in the trade as glucose, dextrose, starch sugar, or as grape or fruit sugar is identical with that found in sweet fruits and honey. On the commercial scale it is produced from starch in closed converters working at pressures ranging upwards from 15 lb. per square inch or in open vessels at atmospheric pressure. The latter method is very slow and conversion is not always complete after ten hours' boiling but the procedure is interesting because the vessels used in brewing may be utilized for the preparation of starch sugar if circumstances should develop that make that expedient necessary. The starch sugar thus produced may be added to a saccharified grain mash for the purpose of supplementing the sugars produced from grain by diastatic activity; it may be mashed alone with malt culms and subjected to the usual souring process, or instead of molasses it may be fermented immediately after its clarification and dilution to store and starting wort.

A starch suspension in water is prepared in the starch cooker and is allowed to flow from that vessel into the mash tun,

where it is boiled with dilute acid until conversion is complete, but in order to avoid an excessive dilution of the syrup with condensed steam the open steam coil is replaced with a closed coil, consisting of eight turns of 2-inch copper tube spaced 4 inches between centres on copper standards that are bracketed 9 inches from the sides of the vat. Steam is admitted at the top of the coil and the water of condensation is discharged through a steam trap placed beneath the vat. The vapour rising from the boiling starch solution is discharged through a flue attached to the cover of the vat.

The starch milk is prepared in the starch cooker by adding raw starch to twice its weight of water and, to avoid the deposition of starch, the mixture is kept in continual motion until the vat is empty. The mash tun is charged with a volume of service water equal to that used in preparing the starch milk; this water is acidified with sulphuric acid corresponding to 4·0% of the weight of starch used, heated to boiling and while it is in active ebullition the addition of starch milk is carefully regulated to avoid any reduction in temperature, but if this should occur the addition of starch milk is immediately discontinued until active boiling is resumed. The progress of conversion is followed by means of the starch iodide reaction and, at the end of the process, by the precipitation of dextrins with 95% alcohol.

The conversion of starch to glucose is far more rapid and complete in pressure vessels. The conversion, neutralization and filtration of the syrup may be completed in the morning and diluted to commence fermentation in the afternoon. There are several types of pressure converters in use. The Henze vertical and Hollefreund horizontal grain steamers often used in distilleries and yeast factories for the gelatinization and liquefaction of raw grain starch before mashing are readily adapted for the purpose; and the Uhland pressure converter is in general use in starch factories for the production of glucose. They all operate on the same principle, however, and differ only in shape and in details such as the composition of the acid-proof lining, arrangement of stirrers, steam jets and mountings. By working at pressures of about 3 atmospheres the conversion is effected in a fraction of the time occupied in open vats, the

syrup is free from the discolouration and defects of that produced in open vats and, according to the dilution of the starch milk, temperature, and amount of acid employed, the syrup will contain a yield of glucose up to 95% of the starch used. This rapid saccharification needs careful supervision so that the process may be stopped at the right moment, otherwise conversion may be prematurely arrested or, if continued too long, the sugar may undergo destruction or reversion to products such as isomaltose.

The process is controlled by means of the iodine reaction until achroo-dextrin and glucose is formed ; a sample of the liquid is cooled and placed either in a test tube or on a white porcelain spot plate with one or two drops of iodine solution, prepared by dissolving 1 gm. of iodine and 2 gm. of potassium iodide in 250 c.c. of water.

The various dextrins give characteristic colours with iodine which affords the best means of distinguishing them from one another, both starch and soluble starch gives a pure blue colour with iodine and as conversion proceeds this changes to the purple colour of amylo-dextrin. In succeeding samples this colour is followed by the red erythro-dextrin tint but the next stage, achroo-dextrin, gives no characteristic colouration with iodine and the disappearance of all the dextrins cannot be determined by this test alone, consequently if a sample taken from the cooker gives only a faint yellow colour with the iodine solution but becomes turbid on the addition of alcohol it indicates that hydrolysis is not complete and that dextrins are still present.

During the conversion process these colours merge into one another and may show that, at one stage, the erythro-dextrins predominate over the amylo-dextrins and at another, that the achroo-dextrins exceed the amount of erythro-dextrins until at the end of the process sugar predominates with from 3 to 5% dextrins and isomaltose. During this stage the samples from the cooker are mixed with large volumes of 96% alcohol and examined for the dextrin precipitate. When this test fails to produce a precipitate the conversion has advanced to such a point that the amount of sugar formed retards the precipitation of dextrin by alcohol. Complete saccharification is then

obtained by continuing the process for some little time after the alcohol test fails to give positive results.

As soon as conversion is complete the acid remaining in the sugar solution is neutralized by the addition of a suitable base to form an insoluble, or sparingly soluble, salt that can be removed by filtration. Lime is the cheapest material available for this purpose, and although an appreciable amount of the calcium sulphate thus formed remains in solution it has no influence upon the ultimate product when the glucose is used for yeast production. Quicklime is unsuitable as a neutralizing agent owing to the formation of coloured decomposition products of glucose even when it is used in slight excess; but calcium carbonate does not share this defect when it is applied in the form of finely powdered limestone, although excessive frothing may be troublesome.

The glucose solution is transferred from the pressure converter to a capacious neutralizing vat that is fitted with efficient agitators that ensure an equal distribution of the limestone in the liquid. A slight excess of the theoretical amount of powdered marble or limestone is taken, mixed to a thin cream and to prevent the limestone becoming trapped in the froth, fed through a funnel and a lead pipe to the bottom of the agitated liquid in the neutralizing vat. Only small quantities are added to the liquid at a time and then at suitable intervals, otherwise the too rapid evolution of CO_2 forms a frothy scum that overflows the vat before it can be dispelled.

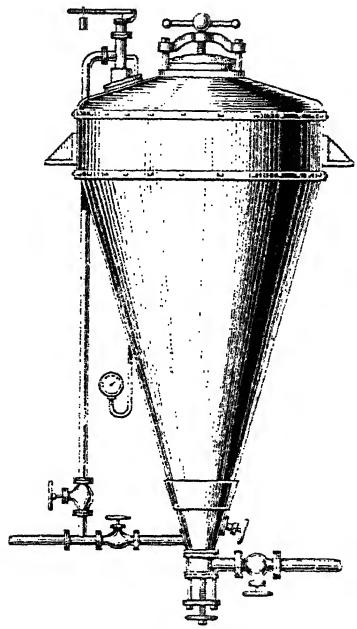
After the acid is neutralized the suspended impurities are removed from the solution by filtration through a small filter-press. With reasonably pure starch this is generally a rapid process, but with crude materials the rate of filtration is always influenced by the amount of debris, other than gypsum and unaltered limestone, suspended in the solution. With the exception of rice, tapioca roots contain a higher percentage of starch than any of the common brewing materials that can be converted to glucose without preliminary treatment and levigation, but unless it is well sifted the fibre content of these tubers retard syrup filtration in a marked degree when compared with syrup prepared from pure starch.

The sediment retained in the filter-press is either washed *in situ* or discharged into hot water, refiltered, and the wash added to the first filtrate. If time permits and the syrup is not required for immediate fermentation it may be clarified more economically by sedimentation; the solid matter is firmly deposited on the vat bottom in from ten to fifteen hours from the strong syrup solution and afterwards in about eight hours from the sediment washing charge.

When it is required for immediate use in a differential fermentation, the hot syrup is pumped direct from the filter-press to the wort cooler. A suitable volume, calculated from its gravity Balling, is discharged into the fermenter and diluted with sterile water to produce the required volume of starting wort at 0·5° Balling and sulphuric acid is added to increase the acidity to *p*H 5·0. The remainder of the syrup is passed into the wort store tank, where it is diluted with weak washings from the sediment to make up the necessary volume of store wort at 12° Balling.

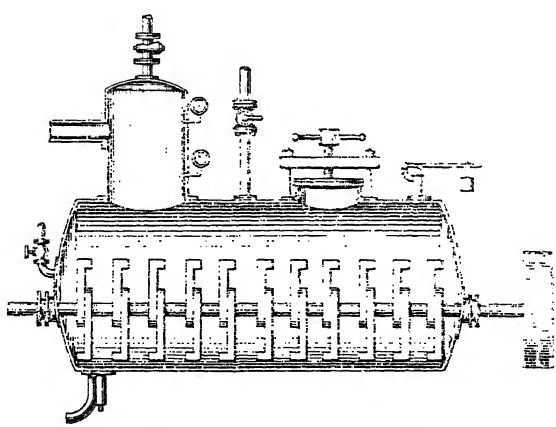
The differential fermentation then follows the normal routine of a molasses brew, with the periodical additions of phosphate solution as previously directed. Potassium sulphate is also required and the additions of inorganic nitrogen compounds are proportioned to maintain a uniform acidity at *p*H 3·5 during the main period of fermentation.

When the glucose solution is used to supplement the sugar content of a grain brew the syrup is applied in exactly the same manner as a solution of cane sugar. The acidity of the syrup is first adjusted at *p*H 3·5 by the addition of sulphuric acid and it is then added to the mash tun immediately after the mash is saccharified, but if the syrup is mashed alone with malt culms it is first diluted to 17° Balling and acidified to *p*H 3·5 in the mash tun. Malt culms are then added in an amount necessary to produce a mash of the normal consistency and soured according to the procedure outlined for an all-grain brew. The amounts of phosphate and potassium solutions required in the subsequent fermentation is determined by analysis and depends upon the proportion of grain or culms used with the glucose syrup.



(*a*) The Henze steamer.

The contents are agitated by the skilful arrangement of a number of steam jets.



(*b*) The Hollefreund converter.

A sectional view showing the mechanical agitator.

FIG. 9. Pressure vessels used for starch gelatinization or acid conversion.

Wort.	Acid.	*p*H.	Formalin, Number
Before Fermentation. .	0·4	4·4	0·2
After Fermentation . .	1·3	3·0	0·15

YIELDS

Yeast.

Kilos. Gross . .	872
less Seed yeast .	172
Kilos, Nett . .	700
Yield per cent. . .	51
Dough test .	51 minutes.
Sugar test .	826 c.c.
Protein . .	

Alcohol.

Concentration .	0·8% by weight.
Kilograms .	142
Yield per cent..	10·3

The volumes of strong wort that are to be delivered to the brew each hour are entered on the ferment chart and the attendant regulates the flow by means of a pointer attached to the stem of the wort plug cock which indicates the rate of flow in litres per hour on a graduated scale attached to the body of the cock; the accuracy of this fitting is checked by observing the movement of the float plummet in relation to the graduations on the wort level indicator board.

The wort contains sugar in excess of that required to produce a 50% yield of yeast, and the formation of alcohol is inevitable, but most of this is dissipated during fermentation and only traces remain in the spent wort. Starting at 78·5° F., the temperature of the fermentation is allowed to rise 0·5° F. per hour during the first seven hours and then at the rate of 1·0° F. per hour until 86° F. is reached, at which it remains until separation commences. In addition to any other instructions that may be necessary the attendant is supplied with a ferment chart similar to that reproduced with the temperatures, air volumes, quantities of inorganic nitrogen and differential volumes of strong wort already entered as directions to be

carefully followed, the gravity Balling of the wort is to be determined and recorded each hour during the fermentation.

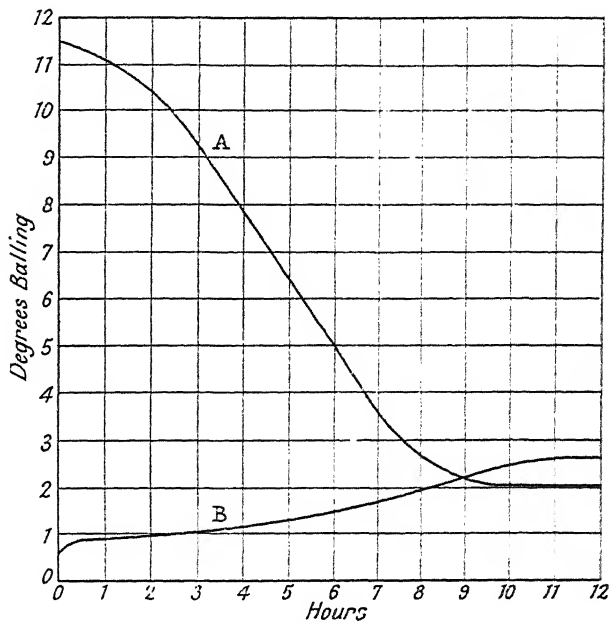


FIG. 10.

A. The falling curve of an 11·5° Balling spirit fermentation. B. The curve of a differential fermentation. The rising curve is principally due to the amount of yeast grown during the fermentation.

CHAPTER VI

YEAST PRODUCTION FROM SPIRIT FERMENTATIONS

BEFORE yeast production became specialized by the introduction of the non-alcoholic fermentation processes developed in Germany during the Great War, commercial yeast was chiefly obtained as a by-product from the alcoholic fermentation of whisky and gin distillery worts. This industry is still a most important source of yeast supply, but owing to the consistently high quality of the yeast produced by the differential processes it is often necessary to standardize the quality of distillery yeast, which varies according to the composition of the wort and the conditions during fermentation, by regeneration in a differential fermentation before it is distributed to the trade.

Repeated attempts to apply yeasts from ale breweries to the bread industry invariably fail owing to the unpleasant bitter flavour imparted to the bread by the resinous bitter principle of the hops precipitated from beer worts during fermentation; which with lupulin glands from the hop flowers remain in the sedimentary beer yeast and cannot be removed without impairing the quality of the yeast.

The processes used in brewing for ale, yeast, and distillery purposes are often classed together, commercially, as if the work and materials were identical, but the differences are of a very fundamental kind. In preparing worts for the distillery and the yeast industry the raw grain is subjected to such treatment with high diastatic malt in the mashing processes that the maximum conversion of starch to sugar is secured. In ale brewery practice the worts for beer are prepared with materials and by mashing processes designed to produce a very high ratio of dextrin to sugar, so that in what is known as the primary fermentation, a considerable amount of the

carbohydrates remain unaltered in the wort and residual sugars are only fermented in a secondary fermentation during storage.

The essential plant in mashing for ale consists of the usual grinding machinery, grist bins to retain the ground malt and other materials until they are required for the mash, and a mash tun which serves for both mashing and filtration processes. This vessel is fitted with a false bottom of slotted brass, or perforated copper, filter plates to retain the grains. A system of drainage tubes and taps are attached to wort escape holes in the bottom of the vat similar to that fitted on the bottom of a filter tank. Cooling coils are unnecessary but steam may be injected at one or more points under the false bottom. A two- or four-arm sparge rotates on the agitator spindle for sparging the grains and an underlet lifts the grains off the filter plates on each occasion before the sparged grains are agitated.

The mash tun is often equipped with a foremasher that mixes the grist with water, at any desired temperature, as it flows from the grain bins to the mash tun. This attachment consists of a horizontal tube about 60 inches long and 12 inches in diameter, down the centre of which an agitator spindle fitted with radial spikes mixes the grist with water and discharges the mash into the mash tun. A hot-and-cold water service is connected to this unit and a slide regulates the admission of grist from the bin.

Another vessel, the starch cooker, is placed in a position commanding the mash tun; this vessel is fitted with an agitator and steam coil and serves for the preliminary treatment of raw grain and other malt substitutes, or when sugar is used to increase the productive capacity of the plant the starch cooker serves as a solution vessel. Malt alone may be used for the grist, but more often malt is employed to the extent of from 55% to 75%, the remainder being barley, maize or rice and sometimes other materials capable of diastatic conversion.

When raw barley or flaked materials are used with malt the mashing process is readily carried through in the mash tun, the action of the malt enabling the barley starch to liquefy and become saccharified at the temperatures employed, but

when maize or other material yielding starch of a refractory nature is used the starch cooker is utilized for their preparatory gelatinization.

Although the mashing methods vary widely in detail nearly all the brews are mashed at temperatures that will produce a ratio of between 45 to 75 parts of dextrin to 100 parts of sugar in the wort. As a rule about half the amount of water necessary to produce wort of the required density is used in preparing the mash, the remainder being reserved for sparging. The ground malt is mixed with hot water to produce a mash at a temperature between 100° F. and 125° F. and the hot decoction of raw grain is added in various ways, the increase in the temperature of the mash is then continued either by injecting live steam, adding hot water, or by heating a portion of the mash.

At 140° F. the maximum amount of maltose is produced by diastase, and to avoid this development the temperature is increased to between 163° F. and 170° F., according to the percentage of unfermentable dextrins required in the beer. The wort is then separated from the solid matters of the mash by filtration and diluted to a suitable density by extracting the mash with hot sparge water.

In whisky or gin distilleries that produce commerical yeast as a by-product the raw materials may consist of malted grain only, but more often raw grain predominates in the composition of the mash. The plant required for the preparation of wort is almost identical with that used in yeast production, and the malt may be prepared from barley, oats, wheat, rye or maize. The chief object in mashing is to secure complete conversion of the starch with as little malt as possible, and for this reason the germination of the malt is carried further than that for ale brewery purposes in order to develop the maximum diastatic power.

When the unmalted portion of the mash consists of barley or oats the starch in these cereals is readily saccharified at the temperatures used in the mash tun, provided that a small quantity of high diastatic wheat malt is included with the barley malt, but the more refractory starches of rice, maize, wheat or tapioca are gelatinized either in the open starch

cooker or more completely prepared for saccharification in an autoclave of the " Henze " type. This vessel is charged with water at 125° F., and after the addition of raw grain air is displaced by advancing the temperature of the mixture to boiling point. The vessel is then closed and the pressure increased to 30 lb. per square inch, at which it remains for 30 minutes ; the pressure is then carefully released, and when the temperature is reduced to 212° F. the gelatinized mash is blown up into the mash tun with steam.

The possibility of affecting the diastase in the malt mash by direct contact with the hot mucilage is avoided by either spraying it into the mash tun against a current of cold air, induced by a ventilating device attached to the mash tun cover ; or the temperature of the mucilage may be further reduced, after releasing the steam pressure, by connecting the autoclave to a vacuum pump and reducing the temperature to a point at which the heat units in the mucilage together with that in the malt will, when mixed, produce a mash temperature of approximately 144° F. The mucilage is then blown into the mash tun with compressed air. When rice or maize is used the latter method is generally adopted, but before being blown into the mash tun it is mixed with 1% of its weight of wheat malt and let stand at 170° F. for fifteen minutes to liquefy.

In the absence of a pressure vessel the unmalted material is gelatinized in an open starch cooker by methods similar to those described in mashing for the differential fermentation of an all-grain brew, or the order may be reversed and raw barley or oats gelatinized in the mash tun and, after cooling to a suitable temperature, the mash is completed by the addition of the malt mash that has been prepared in the starch cooker. This procedure is only adopted when wheat malt is not available and the amount of raw barley that then has to be gelatinized at the highter temperatures exceeds the capacity of the starch cooker, or when time is limited and a more rapid saccharification is necessary than that obtained with wheat malt at the highest mash tun temperature (145° F.) normally used in this process.

The crushed barley and 2% malt is mixed with three times its weight of water at 135° F. in the mash tun, the temperature

is then slowly increased to between 165° and 170° F. and the mash stands for sixty minutes' gelatinization. During this interval a malt mash is prepared in the starch cooker by mixing the remainder of the malt with water at 125° F. The gelatinized barley in the mash tun is cooled to a temperature that when the malt mash is mixed with it the combined temperature is about 145° F.

This method of mash tun gelatinization is applicable to those materials only that gelatinize at relatively low temperatures. Other materials, rice or maize for instance, do not readily liquefy and are cooled with considerable difficulty after gelatinization owing to the mucilage forming a non-conducting jelly that adheres to, and insulates, the cooling coils of the mash tun.

With the exception of the rootlets adhering to green malt, culms are rarely used in distillery mashes and the selection of raw materials is always influenced by their combined filter value. In many cases malted and raw barley is used exclusively but where rice, maize, or similar material is mashed with the spelts only that amount is used that will not interfere with filter efficiency. As a raw cereal in its original form, rice is eminently suited for brewing purposes and, even when stripped of its husk it is a valuable addition to the mash. Its exceptionally high starch content is greater than any other cereal and gelatinizes readily under pressure, but in the open starch cooker special treatment with high diastatic wheat malt is required in order to liquify the starch before it is added to the malt mash.

The crushed rice and 1% of wheat malt is mixed with 3·5 times its weight of water at 130° F. in the starch cooker and, after soaking for thirty minutes, the temperature is advanced to 157° F. for thirty minutes' agitation. By this treatment some of the more friable starch granules are corroded by liquefying enzymes and converted by the wheat diastase, the rice is then heated to boiling point and the longer it is kept at this temperature, within limits, the more completely will the starch be saccharified when mixed with the malt in the mash tun.

The treatment of the distillery mash after saccharification

depends largely upon the procedure then considered necessary in producing the particular type of alcoholic liquor required. High yields of yeast are avoided owing to the corresponding reduction in alcohol that would result, but high yields of yeast could not be obtained even if the worts were aerated owing to the small amount of diffusable nitrogen they contain. The flavour of the distilled liquor is influenced by the acidity of the fermented wort from which it is distilled, consequently the mash is not soured and the alcohol yield is increased by the decomposition of sugar that otherwise would be wasted in the production of lactic acid. It is estimated that the lactic acid bacteria decompose their own weight of sugar in one hour.

The wort being neutral or very low in acidity, bacterial or mould infection would be inevitable unless means of prevention were employed. This is accomplished by the adoption of efficient methods of sterilization, the close supervision of yeast pure cultures, and low temperature fermentation by means of refrigeration. The acidity of the wort varies with the materials used but in a barley mash the acid phosphates, proteins, and traces of organic acids keep *p*H constant, or between very narrow limits, during fermentation.

The process of mashing for yeast production in aerated spirit worts differs from distillery practice in that the wort is not filtered from the grains immediately after saccharification; but instead, the mash is subjected to a souring process occupying from twelve to fifteen hours in order to produce the lactic acid essential during fermentation. The development of this acid is a matter of considerable importance. It retards the growth of infection and is the chief factor in preventing any wide difference in *p*H during the fermentation of the aerated wort; and incidentally, it assists in modifying the proteins during the mashing process.

A higher percentage yield of yeast is obtained from aerated spirit brews than from a distillery mash; and a proportionate increase in the assimilable nitrogen content of the wort is essential. In some cases it may be permissible to use ammonium sulphate for this purpose, but more often this expedient is impracticable and is quite unnecessary when the native proteins are hydrolysed by suitable treatment in the

mash tun. An appreciable percentage of malt culms are always included in the raw material to supply the greater amount of nitrogen required and the filter value of this material allows a higher proportion of raw grain or tubers to be mashed without interfering with wort filtration.

In a differential brew the fermentation is readily controlled by the supplementary additions of suitable chemicals, but in most aerated spirit brews extraneous additions are not desired and the success of the fermentation is determined primarily by the composition of the wort. Thus the yield of yeast and alcohol depends more upon the treatment of the raw materials in the mash-tun than upon fermentation control; and any error in their selection, or in processing, may become unpleasantly apparent in the later stages of the process.

At the end of the souring period the mash is agitated, a few gallons are removed to serve as sour seed for the next mash, the temperature is observed and samples of the wort are taken for gravity Balling and titration acidity determinations. The mash is then sterilized at 160°–168° F. and discharged into the filter tank; the methods of filtering the wort and extracting the grains differs slightly from that described in brewing for the differential fermentation where the mash is discharged into one filter tank and the dilution, equal to fourteen times the weight of the raw material, is so much greater that the volume of sparge water used completely exhausts the spent grains.

With a strong spirit and yeast brew in which the wort is fermented from an original gravity of 12° Balling and the dilution amounts to about 4·5 times the weight of raw material, complete extraction of the grains with the permissible volume of wash water is almost impossible and after the strong wort is diluted to the required density in the fermenter the weak filtrate contains so much extract that it is reserved for sparging the grains of the next brew.

By means of a mash filter-press the wort filtration may be completed in a fraction of the time occupied in gravity filtration and, in addition, the grains are washed and completely exhausted with a volume of water that is much less than that required to dilute the strong wort. In the absence of a mash filter-press, filtration efficiency is considerably improved by the

installation of two filter tanks, each one having sufficient capacity to filter half the mash. The tank from which the wort is first drawn is the only one sparged with water and when the density of the wort filtered from this tank falls 1° Balling from the original it is sparged on to the grains in the second tank.

By this method a greater volume of strong wort is obtained and before the strong wort in the fermenter is diluted to 12° Balling, water only will be flowing from the first filter tank and 1·5° Balling weak filtrate from the second tank. This method may not be as rapid as the straight filtration in one tank but it compensates for the difference in time by the better extract obtained and this may be improved slightly by raking the surface of the grains occasionally to break up any channels that may be formed.

In passing from the filter tank to the fermenter the wort flows over a wort cooler where its temperature is reduced to between 78° and 84° F. according to the temperature at which it is desired to commence fermentation. At normal temperatures the alcohol formed during fermentation checks the activity of yeast in proportion to its concentration, and this effect is intensified by increasing temperatures. For this reason the fermentation of a strong spirit brew commences at the optimum temperature, 86° F., and the effect of the increasing concentration of alcohol upon yeast growth is minimised by progressively reducing the temperature until it reaches 78° F. at the end of fermentation twelve hours later. In this way the reproductive activity of the yeast is maintained but the rate of fermentation is considerably reduced, therefore the time in which the total sugar of the brew is consumed is determined primarily by the amount of seed yeast used and the stimulus it receives from the aeration.

At the temperatures indicated and with the aeration mentioned below, from 2% to 5% of seed yeast will ferment the sugar of a 12° Balling wort within twelve hours, but when smaller amounts are used the fermentation period is extended, resistance to infection is reduced and the quality of the commercial yeast is more easily influenced by slight differences that may occur in the composition of the wort. This feature

is absent when the higher percentages of seed yeast are used and in addition to the uniformly higher quality and yield of yeast produced the attenuation of the wort goes farther and is more complete in the time specified.

The volumes of air injected into a spirit brew require to be as carefully regulated as those for a differential fermentation. If the brew is supplied with too much air yeast growth may be stimulated to produce an amount that exceeds the assimilable nitrogen supplied in the wort, *p*H then varies owing to the dissipation of lactic acid and a slightly higher yield of protein poor yeast is produced with the slate grey tint indicative of degenerate cells, these absorb colouring matter from the wort in the same way that weakened cells are stained with methylene blue. If the brew is supplied with less air than that specified the yield of yeast is reduced in proportion to the deficiency, but unless this is considerable its effect upon the fermentative power and quality of the yeast is negligible.

When fermentation commences with the addition of seed yeast the brew is aerated with a total volume equal to 3 cubic feet of air per minute per cwt. of raw material brewed (0·1 M^3 air per hour per kg. of R.M.). Three hours later this volume is increased to 7·5 cubic feet per minute per cwt. of R.M. (0·25 M^3 per hour per kg. R.M.) until fermentation is finished. At the end of the yeast maturing period, indicated by the gravity Balling remaining constant for sixty minutes, the yeast is separated from the wort and whilst separation is in progress a gentle current of air is passed through the fermented wort to keep the yeast in suspension, otherwise a heavy deposit of yeast may form on the bottom of the fermenter and degenerate by overheating.

The yeast concentrate is chilled after it leaves the separators and is then filter-pressed until the yeast cakes are dry and friable. Owing to the alcohol content of the yeast, which amounts to about four-fifths of that in the wort from which it was separated, and the nutritive effect of the wort still adhering to the cells, the durability of the yeast from a strong spirit brew is improved by washing it in four times its weight of ice-cold water and, after pneumatic agitation for thirty minutes, re-pressing and placing it immediately in cold store.

The indications of the Balling saccharometer are influenced by the amount of yeast suspended in a liquid, 42·56 gm. of yeast per litre of wort equals 1° Balling, and as a check upon the efficiency of separation and filter-pressing operations the approximate yield of yeast in the brew may be estimated from the difference in the gravity Balling of the wort before and after separation :—

$$\frac{42{\cdot}56 \times \text{total litres} \times \text{diff. in deg. Ball.}}{1{,}000} = \text{Gross yield in kg.}$$

The particulars of each brew that are required for comparison and future reference are recorded in tabulated form in a brew book ruled for the purpose. Details of the raw materials and of the progress of the work in each department are entered under appropriate headings in vertical columns, or each brew may be recorded individually on a ferment chart similar to that of a differential brew and filed for future reference. During fermentation the temperature and gravity Balling only are noted at sixty minutes' intervals, there is a considerable reduction in the concentration of lactic acid in the wort, but as *p*H remains nearly constant during the whole period, both acidities are determined before and after fermentation.

The yields of yeast and alcohol are the most important features of the report and if the sugar is determined in the strong wort and calculated as a percentage of the raw material the efficiency of the fermentation may be ascertained by comparing the yield of yeast and alcohol with the theoretical curve on the fermentation graph (see Fig. 11), or the table of theoretical yields of yeast and alcohol. This shows that a 100 kg. mash producing the equivalent of 50% of cane sugar should yield either 90 kg. of yeast or 34 litres of absolute alcohol. A 90% yield of yeast is often produced in the non-alcoholic differential processes but the above yield of alcohol cannot be obtained from any spirit fermentation because other products are formed besides alcohol and CO_2 and an appreciable amount of the sugar is assimilated in the growth of yeast.

The alcohol yield is found by first neutralizing the acids, some of which may be volatile, in a sample of the fermented wort, separating it from foreign matter by distillation and then

ascertaining the proportion of alcohol present in the distillate. In strong alcohol it is often convenient to employ one of the many alcoholometers the stems of which are variously graduated to show densities, or percentages of alcohol by weight or volume, or some other indications that may be interpreted by suitable tables. When this method is employed the percentage error increases as the alcohol concentration decreases and the

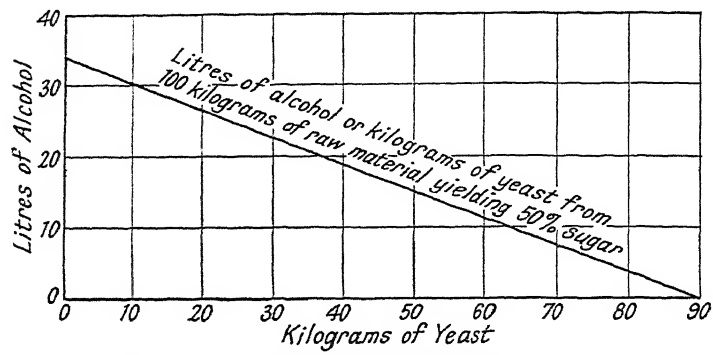


FIG. 11. Theoretical yields of yeast and alcohol.

$C_{12}H_{22}O_{11}$	H_2O	$2C_6H_{12}O_6$	=	$4C_2H_5OH$	$4CO_2$
Cane sugar	Water	Dextrose		Alcohol	Carbon dioxide
342 gm.	18 gm.	360 gm.		184 gm.	176 gm.

100 kg. of cane sugar = 53·8 kg. of alcohol = 68 litres.

density of the weak alcoholic solution distilled from a spirit brew is more accurately determined by the use of a pycnometer as described in "methods of analysis" than by any other means. In recording the alcohol concentration of the wort, some confusion is caused by three ways of expressing the result, as per cent. by weight, per cent. by volume, or in grams per 100 c.c. In laboratory analysis the expression "per cent." implies per cent. by weight unless otherwise qualified; and for the reason that this conforms with most other determinations the weight per cent. plan is the more rational.

Theoretical Yields of Yeast and Alcohol

In percentages from 50 parts of sugar extracted from 100 parts of raw material. Theoretically 50 kg. of cane sugar may be fermented to produce 26·9 kg. of alcohol (= 34 litres)

and no yeast; or to produce 90 kg. of yeast and no alcohol. Thus one part of alcohol is equivalent to 3·345 parts of yeast and one part of yeast is equal to 0·2988 part of alcohol. To compare the yields obtained in practice with the theoretical yields the percentage of sugar in the wort, which may amount to more or less than 50% of the raw material, is determined and the yield of yeast and alcohol then calculated to the 50% sugar equivalent.

Yeast Yield.	Alcohol Yield.		Yeast Yield.	Alcohol Yield.		Yeast Yield.	Alcohol Yield.	
	By Weight.	By Volume.		By Weight.	By Volume.		By Weight.	By Volume.
1·0	26·601	33·52	31·0	17·634	22·21	61·0	8·668	10·93
2·0	26·303	33·14	32·0	17·335	21·83	62·0	8·369	10·55
3·0	26·004	32·77	33·0	17·036	21·46	63·0	8·070	10·17
4·0	25·705	32·39	34·0	16·738	21·08	64·0	7·771	9·97
5·0	25·406	32·01	35·0	16·439	20·70	65·0	7·472	9·41
6·0	25·101	31·63	36·0	16·140	20·34	66·0	7·173	9·03
7·0	24·807	31·27	37·0	15·841	19·96	67·0	6·874	8·66
8·0	24·508	30·89	38·0	15·542	19·58	68·0	6·575	8·28
9·0	24·210	30·51	39·0	15·243	19·20	69·0	6·227	7·90
10·0	23·911	30·13	40·0	14·994	18·83	70·0	5·978	7·53
11·0	23·612	29·75	41·0	14·645	18·45	71·0	5·679	7·15
12·0	23·313	29·39	42·0	14·346	18·07	72·0	5·379	6·76
13·0	23·015	29·00	43·0	14·047	17·68	73·0	5·072	6·39
14·0	22·715	28·62	44·0	13·749	17·30	74·0	4·773	6·01
15·0	22·416	28·24	45·0	13·450	16·92	75·0	4·484	5·65
16·0	22·117	27·86	46·0	13·151	16·57	76·0	4·185	5·27
17·0	21·819	27·48	47·0	12·852	16·19	77·0	3·886	4·89
18·0	21·520	27·10	48·0	12·553	15·81	78·0	3·587	4·51
19·0	21·221	26·74	49·0	12.255	15·44	79·0	3·288	4·13
20·0	20·922	26·36	50·0	11·956	15·06	80·0	2·989	3·76
21·0	20·644	25·99	51·0	11·657	14·68	81·0	2·690	3·38
22·0	20·325	25·61	52·0	11·358	14·31	82·0	2·391	3·01
23·0	20·025	25·23	53·0	11·059	13·93	83·0	2·092	2·63
24·0	19·726	24·85	54·0	10·760	13·56	84·0	1·793	2·25
25·0	19·427	24·47	55·0	10·461	13·18	85·0	1·494	1·88
26·0	19·129	24·09	56·0	10·162	12·80	86·0	1·196	1·50
27·0	18·830	23·74	57·0	9·864	12·42	87·0	0·897	1·13
28·0	18·531	23·36	58·0	9·565	12·05	88·0	0·598	0·74
29·0	18·232	22·98	59·0	9·266	11·67	89·0	0·299	0·37
30·0	17·933	22·59	60·0	8·967	11·30	90·0	0·000	0·00

The approximate yield of alcohol in the brew may be estimated from the difference in the gravity Balling of the wort before and after fermentation. The Balling saccharometer indicates the number of pounds of brewers' extract contained

in 100 lb. of wort. During fermentation the density of the wort decreases owing to the consumption of sugar and the indications of the saccharometer no longer correspond to the real amount of extract contained in the wort. This is owing to the presence of alcohol which, being specifically lighter, allows the instrument to sink lower than it would in a solution containing an equal amount of extract dissolved in water only. For this reason the difference between the Balling indication of the original wort and of the fermented wort is termed the apparent attenuation.

The saccharometer indicates the real attenuation only when the sample of fermented wort is deprived of its alcohol by distillation and its original weight is restored by the addition of water. The difference thus found between the original and the final density of the wort represents the amount of sugar decomposed by the yeast and from this the alcohol content of the wort may be found by dividing the real attenuation by two. Alternatively the alcohol yield may be estimated to the same order of accuracy by multiplying the apparent attenuation by one of the factors, which differ according to the original density of the wort, shown in the table.

Original Density of the Wort in degrees Balling.	Alcohol Factor for the apparent Attenuation.
6·0	0·4073
7·0	0·4091
8·0	0·4110
9·0	0·4129
10·0	0·4148
11·0	0·4167
12·0	0·4187
13·0	0·4206
14·0	0·4226
15·0	0·4246
16·0	0·4267

Seed Yeast for Spirit Fermentations

Seed yeast is always more vigorous and resistant when it is propagated by the large seedings and frequent transfers

necessary for the differential process; but, owing to the lower percentage of seed yeast used in spirit fermentations and the lower yields obtained, it is cultivated by simpler methods in from six to nine generations; the amount of wort in each factory culture increasing by ten or twelve times the volume of the preceding transfer, the number of transfers depending upon the volume and concentration of the routine spirit brews fermented.

A giant culture on wort agar forms the starting point of a seed yeast culture for spirit brews and when the first flask of sweet wort is inoculated from this colony a schedule is drawn up to show the day and time at which the cultures are to be transferred from one vessel to another; the day is also noted when extra raw material should be mashed to provide the wort necessary for the more voluminous factory cultures.

The cultures are fermented at 84° F. in 12° Balling wort and inorganic nitrogen is not supplied unless the wort is deficient in assimilable nitrogen. The penultimate culture and the seed yeast brew are aerated at the rate specified for a commercial spirit brew at temperatures that vary between 82° F. and 88° F., according to the relative yields of yeast and alcohol required. The seed yeast is matured, separated from the fermented wort, and after pressing is washed by aeration for thirty minutes in four times its weight of chilled water at *p*H 4·0; it is then filter-pressed and packed in containers that are clearly distinguished from the commercial yeast.

Double Purpose Brewing

Instead of producing yeast and alcohol in the one fermentation as described in the previous process the quality of each product is greatly improved when the required amount of yeast is produced in a non-alcoholic differential brew and the alcohol is produced alone in a spirit fermentation. By referring to the table of relative yields of yeast and alcohol it is seen that from a brew producing 20% of yeast the theoretical yield of alcohol amounts to 20·9% and there can be no increase in these yields by producing yeast and alcohol in separate fermentations because the same amount of sugar carbon is used in this method as when yeast is also produced in a spirit brew.

The advantage of separating the yeast and spirit fermentations is simply a matter of quality, the yeast is free from alcoholic contamination and the spirit fermentation may be conducted with a minute amount of seed yeast at temperatures that favour alcohol production and retard yeast cell multiplication. The method may be employed without duplicating any part of the plant ; the spirit brew is not aerated and may be fermented in any sterile tank or vat equipped with a cooling coil suitable for controlling the temperature during the fermentation.

A slight reduction in raw material costs may follow when the spirit wort is prepared from materials rich in starch and poor in nitrogen, such as rice or tapioca roots, using malt culms in the differential mash only, but the cheaper wort is also suitable for the differential fermentation and a greater reduction in general costs is effected when the wort for both brews is produced in one mash and fermented simultaneously in separate fermenters.

The volume and concentration of the wort required for the differential fermentation is predetermined by the capacity of the plant and air compressor so that the weight of raw material required for the combined mash depends upon the amount and concentration of alcohol required from the spirit brew. The mashing, sterilization, and wort filtration processes are conducted according to methods previously described, but the division of extract is simplified if the strong wort only is used for the spirit fermentation ; this is drawn from the filter tanks, chilled to 86° F. and discharged into the spirit fermenter until the density of the wort from the filter taps is reduced to 15·5° Balling.

The remainder of the wort obtained from the filter tanks is then used in preparing the store wort at 11° Balling and 60° F., and the starting wort at 9·3° Balling and 78·5° F. for the differential brew. The wort in the spirit fermenter is diluted to 15° Balling by the addition of sterile water, inoculated with an amount of seed yeast equivalent to 0·5% of the weight of raw material mashed, estimated from the extract in the fermenter, and fermented at 86° F. until the wort is completely attenuated. Under these conditions the negligible amount of

spirit yeast produced does not pay for its recovery and the fermented wort may be distilled without further treatment.

Yeast from Weak Spirit Brews

Yeast is extremely sensitive to change in its environment; and its characteristics differ appreciably with the slight differences that occur when it is cultivated in worts produced under identical conditions from the same kinds and quantities of raw material. Consequently the differences in yeast properties are more pronounced when it is grown in worts of widely varying strengths. The most prominent feature, from a commercial point of view, is the difference in yields which vary inversely as the concentration of the wort in which the yeast is grown; if this is diluted to 2·0° Balling before fermentation, yields up to 35% of the weight of raw material may be produced, but in a 12° Balling aerated wort the abundant nourishment at the beginning and the accumulation of metabolic products at the end of fermentation retards the reproductive activity of the cells to such an extent that yields of yeast exceeding 20% are rarely obtained.

In the falling curve fermentation of dilute worts, *i.e.*, 2° to 4° Balling, an immense volume of weak alcoholic wash is produced and its ultimate disposal is determined by local conditions. In districts where very cheap fuel is available the alcohol may be recovered by distillation, but more often yeast production is stimulated to the maximum and the alcohol concentration in the wash is then so low that its recovery is impracticable and the wash is run to waste. The higher yield of yeast obtained from these weak spirit brews and its greater durability and fermenting power, compared with that produced from strong spirit brews, compensates for the loss of this alcohol and, in factories not equipped for yeast production by differential fermentations, a considerable amount of commercial yeast is produced by this simple process.

The percentage composition of the mash for a weak spirit brew differs slightly from that of a strong brew in that the volume of mash water is increased to five times the weight of raw material and some of the extra nitrogen required to produce the greater amount of yeast is supplied by increasing the

amount of malt culms until the consistency of the mash is nearly that of a strong spirit mash. This dilution of the raw material produces a mash tun wort of about 11·5° Balling and hydrolysis of proteins and carbohydrates is far more complete than in thick mashes. The souring of the mash to produce the maximum concentration of lactic acid, its sterilization, and the filtration of the wort follows the routine previously described but the dilution of the wort depends upon the capacity of the fermenter, the density at which it is desired to commence the fermentation and the efficiency of the air machine at high pressures.

The fermenter is usually proportioned so that it contains a dip of 118·2 inches (3 metres) when the wort is diluted to between 2° and 4° Balling. Aeration is most efficient in this depth of liquor and the air compressor is constructed to deliver its rated volume of free air when working against a pressure between 4·5 and 5 lb. per square inch, 6 cubic feet of air per minute per cwt. of raw material mashed (0·2 M^3 air per hour per kg.) are injected into the brew when commencing the fermentation, and after three hours this volume is increased to 12 cubic feet per minute (0·4 M^3 air per hour per kg.) at which it is kept constant until the end of fermentation.

When seed yeast amounting to about 5·0% of the weight of raw material is used to seed the brew, fermentation commences at 80° F. and the temperature is allowed to rise at a uniform hourly rate until it reaches 84° F. at the end of fermentation. Slightly better results are obtained when more seed yeast is used and the fermentative activity of the fermenting yeast is retarded by reducing the initial temperature of the brew.

Worts derived solely from grain and malt culms contain enough assimilable nitrogen to produce a nett yield of 33% yeast, and if this percentage is not exceeded the lactic acid produced in souring the mash will keep the acidity of the wort at about *p*H 3·5. Titration acidity diminishes from the start to the finish of fermentation owing to the gradual dissipation of lactic acid and in order to ensure the optimum conditions for yeast growth the formalin number and *p*H are determined in the wort at intervals of two hours during fermentation and any deficiency in nitrogen and acidity then found is

rectified by the addition of suitable quantities of ammonium phosphate or sulphate.

The details of each brew and its fermentation are recorded on a ferment chart that is filed for future reference; and as the formalin number and *p*H of the wort may be adjusted to any value required, in addition to temperature control, the fermentation is more easily controlled and the record more complete than that of a strong spirit brew. Very dilute worts are completely attentuated at the end of fermentation and the separation of the yeast, its chilling and filter-pressing, follows the same procedure as that described in the differential fermentation of an all-grain brew.

CHAPTER VII

DRIED YEAST AND YEAST FOODS

COMPRESSED yeast in the commercial condition is just as sensitive to temperature changes as it is in the fermenter, and when it is exposed to unfavourable climatic conditions for any length of time the autolytic endo-enzymes attack the cell plasma and the yeast is decomposed by a process known as autolysis (self-digestion). This feature is always a serious problem in the distribution of fresh yeast to distant consumers or to those beyond the range of refrigerator cars, and although these transport difficulties were considerably reduced by the introduction of the more durable product of non-alcoholic fermentation processes, the distribution of yeast in sparsely settled tropical countries is almost impossible except in a dried form.

So-called dry yeast cakes have been prepared for many years by first mixing a little yeast into a stiff paste with wheat meal, starch, or a similar porous and absorbent material. This is cut into cakes, sun-dried, packed in air-tight containers and sold as dried yeast cakes, but the preparation can only be regarded as a very impure and uncertain starter ; the amount of yeast it contains is only a minute fraction of the mixture, and the number of yeast cells may not exceed that of foreign micro-organisms.

In yeast desiccation the experience of many of the earlier investigators indicated that the durability of dried yeast was greatly influenced by the medium on which it was placed for drying. For instance, yeast mixed and dried with powdered charcoal will retain its vitality for as long as ten years, and Hansen found that when it is dried on cotton-wool some of the cells will live for over three years, but if it is dried on a metallic surface yeast will live no longer than three months. These experiments demonstrated that it was possible to preserve

yeast by desiccation and to keep it for long periods without any reduction in its fermenting power. The use of such desiccating agents was undesirable, however, and prevented the industrial application of dried yeast in this form except as a starter, and efforts were concentrated on the development of a process for drying yeast without any extraneous addition.

Yeast from spirit brews of different strengths were used at first in these investigations, and no difficulty was experienced in drying the yeast without immediately destroying its fermentative power, but it was found that its durability differed widely according to the original density of the wort in which it was grown. Yeast cells from strong spirit brews were all dead in from two to three months after drying, whilst some of the cells from the more strongly aerated thin spirit brews were still alive after six months. Yeast cells deprived of moisture in this way do not all die at once nor do they all die immediately after a given time, but their power gradually diminishes, and the number of cells dying in a unit of time is proportional to the number surviving.

Before it was known that the original density of the wort in which yeast was grown exercised such a powerful influence upon the quality of dried yeast, wide differences in its fermenting power and durability were attributed to other factors such as the extension or the reduction of the drying period due to variation in atmospheric humidity and the amount or thickness of the layer of yeast in process of drying; but a series of carefully standardized experiments demonstrated that although uniformity in conditions during the drying process was desirable, the effect of any reasonable variation in the drying period was very slight; therefore, the installation of a general air-conditioning plant was quite unnecessary, but the fact was definitely established that the quality of desiccated yeast is determined by the conditions under which the fresh yeast is grown.

The yeast cultivated by non-alcoholic fermentation in worts derived solely from grain and culms produces dried yeast that retains its original fermenting power for over twelve months and if kept cool and dry it will be found to contain many vigorous cells after three years. Yeast produced from

molasses worts by the same process is much less resistant than the above, but it is far superior to any of the spirit yeasts which prove to be more unsuitable for drying purposes as their alcohol content increases.

Plant Required for Drying Yeast

The plant necessary for drying yeast consists of two essential units, a machine for extruding the fresh yeast into long strings of uniform thickness and consistency, a dehydrator in which these strings are dried on wire screens, and sundry utensils and air-tight bins necessary for the storage, weighing and packing of dried yeast in cans for distribution.

The extruding machine may be especially designed for the purpose, but excellent results are obtained by fitting a copper plate, perforated with $\frac{1}{8}$ inch holes at $\frac{1}{4}$ inch pitch, over the delivery end of a yeast-pounding machine or power mincer. The fresh yeast is moistened if necessary, kneaded until it has the right consistency and fed into the hopper of the machine, from which it issues in long strands that neither break or stick together. When strings of this consistency are produced no difficulty is experienced in spreading them rapidly and uniformly on the drying screens, but if the fresh yeast is too dry the strings disintegrate in spreading, and powdery yeast falls through the screens to collect in heaps either upon screens below or upon the floor. If the fresh yeast is mixed with too much water before "stringing" it cannot be worked because the strands either stick together as they issue from the machine or bunch in lumps when attempts are made to spread them upon the screens.

The dehydrator (Fig. 12) is a tunnel-like structure 6 feet high, 6 feet wide, and 22 feet 6 inches long (internal measurements), situated at as high an elevation as possible in the yeast factory in order to ensure an abundant supply of fresh air. The walls and cover are constructed of 1-inch tongue and grooved timber nailed to 3-inch uprights and lined with sheet iron, or similar impervious material, made reasonably airtight by filling the cavity with granulated cork or sawdust. Three close-fitting doors, each allowing a clear opening of 40 by 72 inches, are

provided on the working side of the dehydrator through which the three portable racks of drying screens enter and leave the drying chamber. A smaller door gives access to the chamber between the air diffusion screen and first portable rack for observation and drying control purposes. The draught inlet is situated at the extreme end of the chamber opposite the fan and is fitted with adjustable louvres and baffle plates designed to distribute the draught equally over the steam coils. The fittings in the dehydrator consist of a multivane fan capable

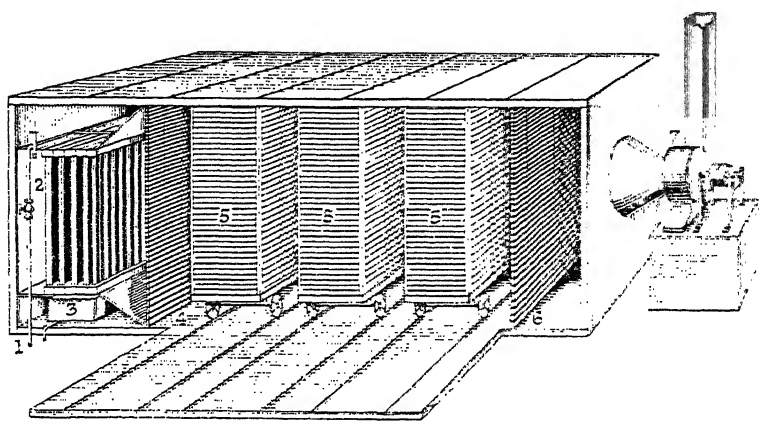


FIG. 12. The dehydrator with one side removed to show the internal fittings.

1. Steam pipe fitted with pressure reducing and control valves. 2. Steam coils, heating the incoming air. 3. Steam trap. 4. Hot air diffusing screen. 5. Drying tiers. 6. Exhaust diffusion screen. 7. Exhaust fan.

of exhausting 9,000 cubic feet of air per minute, three portable drying racks with screens, steam coils, draught diffusion screens, a thermo-regulator, a recording thermometer and a hygrometer.

The air attemperating device takes the form of steam coils arranged in three tiers, each containing thirty horizontal steam pipes 45 inches long and 1 inch in diameter. The coils may be made of pieces of steam pipe cut to length and fitted together with return bends, but the possibility of one or more leaking joints saturating the drying chamber with steam is avoided if the coils are made without fittings from lengths

of steam pipe welded together and bent to form each tier with a 1-inch vertical space between each tube. The tiers are spaced 6 inches apart and connected at the bottom by means of a tail pipe to the steam trap and drain. The thermo-regulator admits steam to the coils through diaphragm valves that are adjusted to maintain the temperature of the drying chamber at 88° F. during the first hour and at 90° F. for the remainder of the drying period.

The two diffusion screens are made of 1-inch square wooden slats suspended horizontally from the ceiling at each end of the drying chamber on cords passing through holes bored diagonally at the ends and middle of each slat (see Fig. 13). The distance

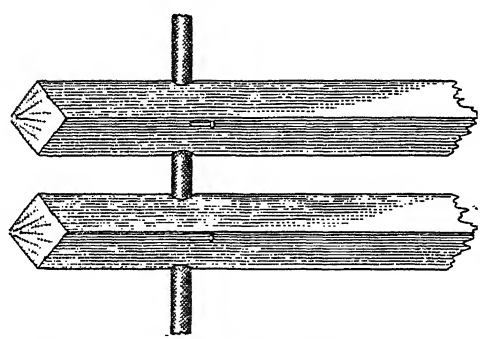


FIG. 13. A section of the diffusion screens.

separating the slats increases progressively from $\frac{1}{8}$ inch at the top of the chamber to $1\frac{1}{2}$ inches on the floor. The multivane fan is placed on the outside and at the end of the dehydrator opposite the draught inlet, and may be either belt driven or direct coupled to an electric motor. It has a cubic capacity of 9,000 cubic feet of air per minute and induces a draught with a velocity of 4 feet per second through the drying chamber.

The portable racks are angle iron frames 66 inches high, 36 inches wide and 68 inches long, inside measurement, mounted on a sheet metal plate and small flanged truck wheels that travel in and out of the drying chamber on sunken rails. The racks are arranged side by side with the distance of the door frame only separating each rack. Six 2-inch angle iron

uprights, gusseted at the top and bottom to ensure rigidity, support the thirty ¾-inch angle iron slides upon which the two tiers of drying screens rest, each screen being separated by a vertical distance of 1 inch, a spacing that assists in securing an even distribution of the draught.

The 180 drying screens required to fill the racks are made of 12-mesh wire gauze tightly stretched and attached to the inside edge of flat iron frames 36 inches long and 32 inches wide, formed by welding suitable strips of 1 inch by ⅛ inch flat iron (see Fig. 14).

The dials of the thermo-regulator and recording thermometer are grouped together on the outside of the dehydrator to

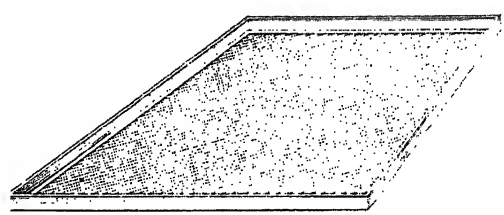


FIG. 14. Framed wire gauze screens.

facilitate temperature control. The installation of a recording hygrometer is of statistical interest only because humidity control is not only costly but has no appreciable influence upon the quality of the dried yeast. An indicating hygrometer, wet and dry bulb, placed in front of the first yeast rack inside the drying chamber gives all the information required to forecast the time occupied in drying the yeast, and this is governed by the relative humidity of the atmosphere.

The quantity of water vapour present in the atmosphere varies greatly, but the drying effect of the air depends more on how nearly the atmosphere is saturated with moisture than on the absolute quantity of water vapour contained in a unit volume of the air. Owing to the fact that the vapour tension of water increases rapidly with the temperature the quantity of moisture which at low temperature would saturate the air will at higher temperature produce quite a low humidity. Fortunately the relative humidity of the atmosphere varies

widely at intervals of a few hours during the day and only on very rare occasions is it high for periods long enough to extend the time required to dry the yeast beyond four hours.

The moisture content of dried yeast prepared from different brews is not always the same, but may vary between 7% and 10%, even though the fermenting power is uniform. Fresh compressed yeast contains 73% moisture; therefore the average quantity evaporated in the drying process amounts to 64·5%, and as each drying screen is spread with 20 oz. of fresh yeast, a total of 225 lb. for the three racks, the output of the dehydrator, including loading and discharging time, amounts to 80 lb. of dried yeast at intervals of six hours.

The Drying Process

The drying process commences by "stringing" the yeast, and as the strands of fresh yeast are discharged from the machine they are caught upon a sheet metal slide in lengths of about 18 inches and distributed upon the drying screens in a single layer with each strand spaced to expose its full surface area to the drying air current. Irregular drying results at the point of contact, and the quality of the bulk is reduced, if a small bunch of yeast strands, which dry on the outside only, is allowed to pass unnoticed into the dry yeast bin. As soon as the first rack is filled with the yeast-covered screens it is run into position nearest the steam coils in the chamber and its drying commences immediately. Evaporation is rapid during the first thirty minutes, and if the racks are placed in position in the order in which they are filled the draught flowing over them is not saturated with moisture when the second and third racks are placed in their positions at intervals of about thirty minutes.

The course of the drying is easily followed by the colour assumed by the strands as the yeast is deprived of moisture. The ivory-tinted threads quickly bleach under the influence of the draught and the more exposed points change to a light brown; this tint rapidly spreads over the whole surface until the strands become case hardened and may be lifted intact from the screens. As drying proceeds the strands shrink

slightly and the core of white yeast, seen on breaking a strand, grows smaller and finally disappears. There is no scientific test that can be rapidly applied to show when the yeast is dry; the conditions of the yeast strands are the only effective indication and the racks are removed from the drying chamber about fifteen minutes after the biscuit-coloured strands become brittle and quite friable.

The greater part of the dried yeast falls off the wire screens when they are held vertically, but a small percentage of the strands adhere to the wire in drying and this is dislodged by means of a soft wire brush. The yeast falls directly into the bins from the screens and, when required, is prepared for sale by grinding to a coarse meal in a small power grinder and sold in airtight containers as pure dried yeast. Satisfactory results may be obtained by the consumer if the dried yeast is taken from the can and mixed with the dough in that condition, provided that the dough is well kneaded to distribute the cells as they absorb moisture, but as the yeast was deprived of its original moisture during a period of about four hours, the best results are obtained when the yeast is restored to its original condition by allowing it to absorb moisture for a similar period before use. For this purpose the amount of yeast required is removed from the can to a shallow dish, sprinkled with water and left covered with a wet cloth until it assumes its original consistency and colour.

Owing to the different methods employed and the uncertain technique of household breadmaking the results obtained in this industry are more satisfactory if the dried yeast is mixed with one half its weight of wheat malt flour to produce a dried yeast compound that is packed exclusively for the household trade; the addition of the malt flour not only acts as a yeast food and bread improver, but facilitates moisture absorption when the dried yeast is mixed with water. Wheat malt flour may become contaminated with the eggs of one of the species of the flour moths from infested conveyors or grain bins, and to avoid their incubation and the development of larvæ in the cans of dried yeast compound it is necessary to heat the sifted malt flour to 150° F. for ten minutes in a mechanical mixer and then mix it with the dried yeast after cooling.

Yeast Foods

The introduction of compressed yeast as a leavening agent not only effected a marked improvement in the quality of bread in which it was used, but it was primarily responsible for many of the revolutionary changes in the processes used in the industry.

The natural desire of the producers to increase sales and to overcome the antipathy of conservative bakers to the use of compressed yeast made it necessary for the producer to ensure that the bread produced by his customers was uniformly superior to that made by users of other yeast. To achieve this result attention was focussed upon the complex problems attending breadmaking.

The Influence of Yeast Foods in Dough-making. Until recently there was probably no manufacturer less aware of the chemical reactions and biological principles involved in the process of his trade than the master baker, many of whom in this modern age use the same unchanging methods in the art that were in use in ancient Rome.

The yeast producer quickly realized that his interests were intimately related to the quality and nutritive value of the loaf and initiated research in this, the last branch of the fermentation industry, to commence a systematic study of the materials used and the processes which constitute the trade.

It was soon found almost impossible to predetermine the quality of the bread that would be produced from any particular flour, and that the question of baking value was extremely complicated owing to the fact that what is termed strength in flour represents the result of the co-operation of many different factors and flour constituents, the most important of which is related to the gluten and its treatment in doughing and fermentation.

Gluten consists of a more or less loose association of two protein bodies, peculiar to wheat flour, known as glutenin and gliadin. When moistened with water glutenin swells slowly to a sponge-like mass; gliadin swells rapidly and forms a colloidal suspension which is immediately absorbed in the fine capillaries of the sponge-like glutenin, producing the tough

and almost indigestible substance, with entirely different properties, known as gluten.

The art of dough-making is mainly concerned in modifying the gluten to an easily assimilated food, extensible in the dough and yet having sufficient toughness to retain the carbon dioxide formed from the sugar during the fermentation of the dough. It appears that there is no relation between the quantity of gluten and what is known as the "strength" of flour; but the quality of the gluten is of the utmost importance, and this is determined in a measure by the ratio of glutenin to gliadin. Its extensibility is profoundly influenced by the subsequent treatment of the flour in dough-making and its fermentation.

Preparation of Yeast Foods. The presence of fermentable sugar in the flour is essential if fermentation is to proceed at the vigorous rate demanded in bread production. Sound flour normally contains about 1·25% of sugar, mostly sucrose supplemented by traces of maltose derived from the flour during fermentation. This quantity is not sufficient to yield the CO_2 necessary to leaven the dough, but any deficiency in the rate of gas production can be supplemented far more readily than can other deficiencies that may be manifested by the flour.

In many of the bleached flours diastase is almost non-existent, and this defect may be rectified by adding malt extract or diastatic malt flour to the dough; but many bakers are not capable of adjusting deficiencies of this kind with any degree of accuracy, and therefore have no desire to superimpose diastatic preparations on their basic dough formula.

Sufficient scientific work has been done for the industry to indicate the complexity and variety of the problems awaiting solution, and considerable advance has been made in knowledge of the gluten and the factors which influence its transformation under varying conditions, such as proteolytic enzyme action, moisture absorption, colloidal properties, the hardening action of salts, etc., but the greatest technical advance is the widening application of the theory of ionic dissociation to breadmaking.

Sorensen definitely proved that the *p*H of dough is of primary importance in determining the quality of bread by its influence upon enzyme activity and the development of yeast cells and its effect upon the modification of gluten and other cereal

proteins. He proved that the quality of bread rises until the hydrogen-ion concentration reaches about *p*H 5 in the dough and that the quality falls in proportion as *p*H diverges from this point.

The Kansas State Agricultural College (*Bulletin* 190, p. 250, 1913) published the following tabulated results of a series of experimental baking trials in which various amounts of ammonium chloride had been mixed with the flour from which the dough had been made.

Grams of NH_4Cl used per loaf .	None	0·025	0·1	0·2	0·4
Time for first rise . minutes	60	60	60	63	62
Time for second rise ,,	75	75	71	63	62
Time for third rise . ,,	22	20	17	16	14
Total time for proving ,,	157	155	148	142	138
Maximum amount of second rise . . . . cm.	24	24·5	23	23	22·5
Loss of material before baking gm.	43	49	43	44	42
Spring in oven . . cm.	3·3	4·2	5·3	6·1	6·1
Volume of loaf . . c.c.	1,300	1,420	1,520	1,600	1,610
Weight of loaf . . gm.	432	437	434	436	437
Condition of crust percentage	93	93	93	93	93
Condition of crumb ,,	96	97	95	98	99

The significance of the result of this experiment was immediately appreciated, and the relation between the presence of electrolytes in the dough and transformation of the gluten was closely studied. It was also found that during the fermentation of the dough the requisite amount of NH_4Cl was dissociated to supply the yeast with nitrogenous nutriment and that the hydrochloric acid thus liberated effected an adjustment of *p*H to near the optimum.

This effect may be investigated in a very simple manner by means of a solution of methyl red indicator, which has a *p*H range from *p*H 4·4 to *p*H 6. The average dough lies well up in the acid and red end of this range.

A convenient method of estimating the *p*H of the dough is to put 1·5 c.c. of the indicator solution in each depression of a white porcelain test plate and at dough mixing and at intervals thereafter place a little ball of dough in the indicator; as fermentation proceeds the acidity of the dough increases, producing a deeper shade of red in the indicator solution.

The introduction of numerous proprietary bread improvers quickly followed the publication of the results of these investigations, and amongst the materials present in many of the preparations may be found ammonium salts, phosphates, calcium compounds, bromates, salts of peracids and other substances that have long been known to accelerate yeast activity; but the usefulness of a great number depends upon a deficiency in the ordinary flour of the substances which they contain.

Provision must be made for adequate nutrition of yeast cells during the fermentation of dough; and what may be termed a standard yeast food and flour improver is composed of a mixture containing 2 oz. of ammonium chloride and 7 oz. of diastatic wheat malt flour. When this quantity is added to 100 lb. of flour it gives even a weak flour the necessary strength to be converted into well-piled loaves with a very even crumb texture; and in addition it increases the yield of bread and physical extensibility of dough made from strong flour.

CHAPTER VIII

THE YEAST FACTORY

YEAST factories are frequently housed in disused breweries, distilleries or any other suitable building that can be adapted to the purpose. Consequently the vessels and other units of the plant are placed in the most convenient positions and economy in operation is often subordinated to expediency, with the result that there are almost as many schemes of plant layout as there are yeast factories ; but one feature that appears common to nearly all is that the mash tun is superimposed on the filter tanks.

Plant and Fittings

Fig. 15 shows one method of arranging plant that combines economy in operation with convenience in supervision. It is not suggested that this particular layout should be strictly adhered to but it shows the disposition of the vessels and other units arranged to give the most economical service in the smallest compass. It would be possible to arrange the plant so that only grain need be elevated, the products descending from floor to floor through the various stages of manufacture until yeast alone would be delivered on the ground floor, but such a scheme would be both costly and inconvenient.

On the other hand, the whole of the plant may be disposed on the one floor, the material being mechanically transferred from one vessel to another in the process of manufacture ; and in buildings where the necessary height is lacking for gravitational discharge this expedient is adopted to transfer the gelatinized raw grain from the cooker to the mash tun, or to discharge the contents of the mash tun into the filter tank, or to elevate the yeasty wort to the separator feed pipes when the fermenters are situated on the ground floor. In designing a building to house a yeast factory it is generally constructed so

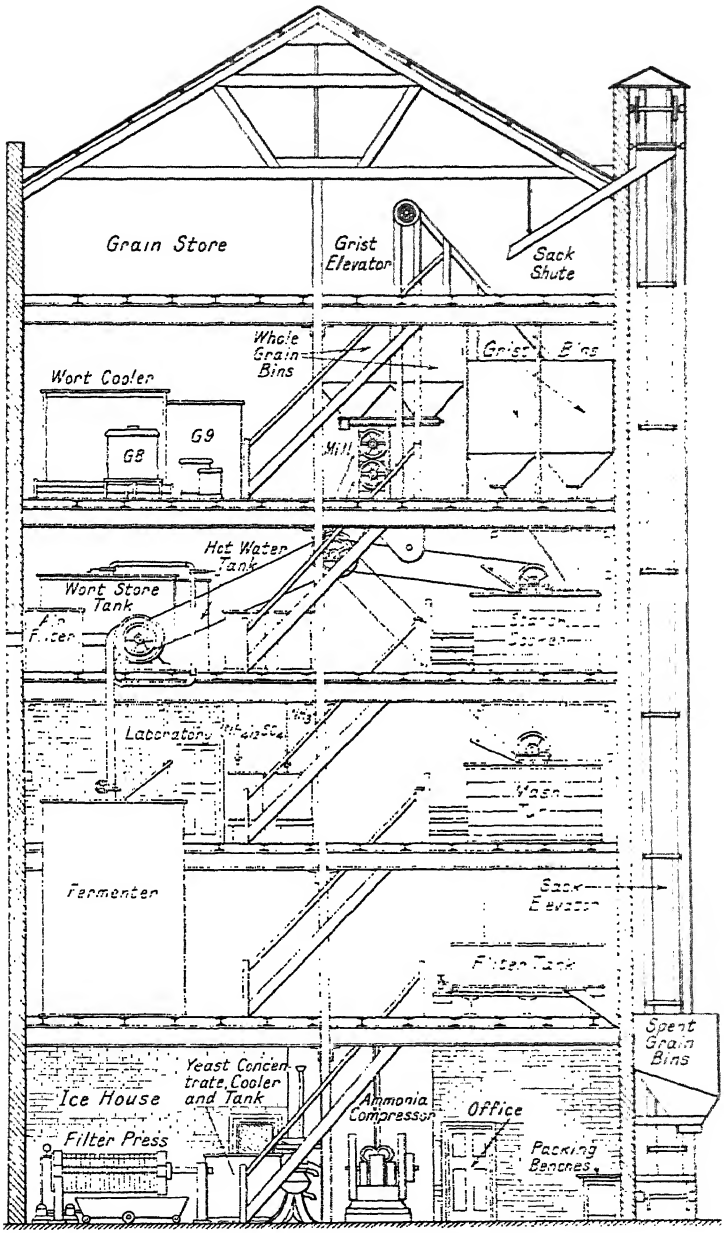


FIG 15. The yeast factory.

owing the disposition of the plant and fittings arranged on the gravity plan.

that the raw materials and the hot wort from the filter tanks only are elevated to the higher parts of the building and they descend by gravitation from these points in progressing from one stage of manufacture to the next.

The raw grain and malt are elevated by means of a simple bag elevator to the top floor of the building and the amounts required for each brew are tipped direct into the grain bins, separate bins being reserved for malt, raw cereals and tapioca. The latter material is reduced in size to that required for it to pass through the feed rolls of the mill by a preliminary crushing in a coke crusher or similar appliance before its addition to the grain bins. This reduction is accomplished by discharging the roots on to a bench about 30 inches high and hand feeding the tubers into the hopper of the crusher, at the same time separating the stones and debris that is often found amongst the roots. The crusher is placed in the grain store and by opening a screw controlled slide the crushed root falls from the crusher into its bin.

Four bins at least are required; one each for the whole grain and the milled grain and the same for the malt. The material gravitates from the whole grain bin to the mill hopper and after passing through the mill rolls the meal drops into the boot of a screw conveyor and is delivered into the grist bins situated directly above the brewing vessels. This arrangement may be modified and construction costs reduced by installing a bucket elevator to remove the grist from the mill and deliver it to any other point desired; the grist bins may then be placed upon the mill floor alongside the whole grain bins and the height of the building correspondingly reduced.

The hot water required for brewing and for general purposes is contained in vessels placed at one of the highest points of the factory so that it gains in velocity in gravitating to the various parts of the building.

The layout illustrated may be termed a modified gravity system in that the solids are elevated to the top floor of the factory, descending until the residues are discarded. The raw grain meal gravitates from the grain bins to the starch cooker and the mash tun is similarly charged with malt meal and gelatinized raw grain. The complete mash then gravitates

to the filter tanks and the exhausted grain falls into the spent grain bins.

The liquid extract from the grain is elevated and descends in a similar manner until it is discarded. The wort is elevated to the wort cooler and gravitates to the wort store tank and fermenter. The latter vessel is situated either upon the first floor or sufficiently high for the fermented wort to flow through feed pipes and sieves to the separators placed upon the ground floor, and from these machines the spent wort is either discharged down the drains or otherwise disposed of.

The finishing process commences at this point and pumps are necessary to elevate the yeast concentrate to the top of the yeast cream cooler. This unit is placed in such a position that it is clearly visible because the condition of the yeast cream sometimes indicates the treatment necessary in the filter-pressing; voluminous froth indicates infection but its absence does not indicate yeast purity.

The yeast cream flows from the cooler into the filter-press supply tank. The filter-press is situated near the separators and retains the yeast as solid cakes that are afterwards discharged into suitable receptacles in which it is mixed with chilled water to the commercial consistency before its storage in the ice house.

Statements have been made claiming that the high pressures and mechanical friction to which compressed yeast is subjected in the yeast factory have an injurious effect upon the cells and that many of the dough fermentation troubles experienced by the baker may often be traced to this influence.

This feature was investigated in a series of carefully checked tests in which it was demonstrated that there was no difference in the dough-raising power, attenuation, fermentative energy, durability, reproductive power or microscopic examination between the check sample of yeast filtered from the wort and tested; and samples of the same yeast that had circulated as a 5% solution through a centrifugal pump for sixty minutes, through a reciprocating pump for the same period, was then filter-pressed, subjected to pressures from 3 to 6 tons per square inch and then tested.

The Sack Elevator. Sacks of grain are most economically

elevated from the ground to the higher levels by means of this unit. It consists of two endless chains operated by sprocket wheels spaced about 3 feet apart and between them support horizontal wooden or steel bearers at intervals of 10 feet or more, according to the height of the elevator and the breaking strain of the chains. Sacks of grain are placed upright against the back board upon loading platforms and are then pushed upwards by the horizontal bearers until they reach the tipping roller, against which they are tilted by an inclined section of the back board.

Bucket Elevators. The most practical method of transporting loose grain or grist from one part of the factory to another is by means of the bucket elevator. This appliance consists of steel buckets shaped according to requirements attached at equal distances to a webbing belt or endless chain; in turning round a sprocket wheel placed at the lowest point of their travel the buckets dip into a grain receiver known as the elevator boot and scoop up the grain or grist; they then pass upwards and around a small sprocket where they are inverted and the contents are thrown out to discharge into a chute or bin as desired.

Screw Conveyors. The screw conveyor is an appliance used to move and deliver grain from a horizontal direction; it consists of a wooden trough containing a closely fitting spiral screw, or separate blades attached to a shafting in such a manner to form a spiral screw. This revolves around its axis and pushes the grain with its blades in the direction of the spiral movement.

Grain Bins. When the materials selected for each brew are weighed they are discharged through tipping hoppers into these receptacles and, after milling, the grist is returned or discharged into bins for convenient storage. Grain bins may be made of either wood or sheet steel on braced angle iron frames; lower maintenance charges and the fireproof qualities of steel construction compensates for its slightly higher initial cost. The bins are generally square in shape with an inverted pyramidal base to insure complete discharge of the contents; access is given by a manhole situated either at the top or side and the bins are usually closed at the bottom with a screw-

operated slide by means of which the rate of discharge is controlled.

In estimating the dimensions of bins necessary for definite amounts of crushed grain it is customary to ignore the capacity of the pyramidal base at the bottom because a corresponding space at the top of the bin remains unoccupied owing to the grist piling to a cone shape as it is delivered from the chute.

The capacity of bins required may be estimated from the tabulated weight of cubic feet of raw materials.

Materials.	Whole Grain.	Crushed Grain.
Maize	50 lb.	34 lb.
Rye	48 ,,	36 ,,
Barley, Chev.	39 ,,	24 ,,
Tapioca roots	—	29 ,,
Wheat	48 ,,	32 ,,
Rice	40 ,,	31 ,,
Wheat malt	40 ,,	30 ,,
Barley malt	32 ,,	23 ,,

The Grain Mill. A wide variety of raw material is used in yeast production, and nearly every kind of material differs from the others in some feature so that it requires different milling treatment in order to obtain the best results in brewing. Fine grinding would be advisable if the results desired were simply a matter of starch gelatinization and hydrolysis, because a complete conversion would be rapidly effected owing to the greater surface of the starch granules exposed in mashing, but a perfect conversion is of little value if the extract thus produced cannot be filtered from the grain residues and the filtration efficiency of the mash is always a matter of prime importance.

For this reason the raw material must be milled in such a manner that the husk or epidermis of the grain is preserved to assist filtration whilst the starch body is fully exposed to treatment in the mash. The full range of raw material may be utilized if the milling equipment includes a coke crusher for breaking tapioca roots, an emery grinder, or an attrition mill for grinding maize and a roller mill fitted with two pairs of

rolls, the upper pair being corrugated and the lower pair cut with fine grooves. The corrugated rolls are opened wide enough for friable cereals to pass through almost untouched, because this grain is efficiently milled by the bottom rolls only; but when tapioca root is in process the corrugated rolls are closed in order to reduce the lumps to a size suitable for the bottom rolls to grip and complete the crushing.

The Starch Cooker, or "Maize Vat." This vessel is usually placed in a position that commands the mash tun so that its contents are discharged into that vessel through a short length of wide-diameter pipe. The starch cooker is a wood stave vat fitted with covers, a mechanical agitator, a perforated coil for the admission of live steam, a 2-inch water service and a small bib-cock and hose for cleaning purposes.

The contents of the vat are discharged through a tube of suitable length screwed into a 3-inch flanged plug cock bolted to the bottom of the vat.

The dimensions of the vessel depend upon the relation of the depth of the contents to agitator efficiency, and it is found that the best results are obtained when the diameter of the vat is twice the depth of the mash. For the purpose of treating the raw grain and starchy materials required to produce the mash of 27 cwt. previously described, the starch cooker has a diameter of 96 inches (243·8 cm.) and a depth of 54 inches (137·1 cm.). This depth allows 6 inches freeboard above the level of the contents. The vat is closed with sheet metal covers stiffened externally by riveting strips of tee iron to the top of the cover and 2-inch angle iron around the circumference.

The covers are hinged in two segments on both sides of a 15-inch channel steel gear support that spans the top of the vat. On one cover a grain chute seats in a suitable aperture and both covers are counterpoised to facilitate opening and closing.

The vat may be made of softwood such as douglas fir or cypress pine, or hardwood such as oak, tuart or mountain ash. The staves are 5 inches wide backed and hollowed from $3\frac{1}{2}$-inch timber, $60\frac{1}{2}$ inches long with a 4-inch chimb and a croze $2\frac{1}{2}$ inches wide by $\frac{3}{4}$ inch deep. The hoops are made of 1-inch iron rod, bolted into 8-inch shoes and spaced on the vat at 12-inch centres with an extra hoop covering the croze. The bottom

of the vat is made of 3-inch timber dowelled every 3 feet at the joints and chamfered round the edge to fit tightly into the croze.

The agitator consists of two wooden blades cut diagonally from 6-inch square hardwood, bolted to a central cast-iron bracket and cut to an overall length of 7 feet. The bracket is keyed to a central spindle of 2-inch shafting rotating in a flanged footstep bolted to the centre of the vat bottom. The agitator blades revolve with a peripheral speed of 700 feet, or thirty-two revolutions, per minute. Power is transmitted to the agitator from the top of the vat by an 18-inch crown wheel keyed to the agitator spindle and driven by a 6-inch pinion. The pinion shaft, fast-and-loose pulley and striking gear are supported on brackets bolted to the channel steel on which the vat covers are hinged.

About eight horse-power is required to start the agitator from rest under full load, but when this is in motion no more than three horse-power is required to continue the agitation.

The admission of steam to the coils is controlled by a valve placed at the top of the vat; a 1-inch copper tube connects this to the coil by piercing the gear support and descending to the bottom where it is teed to the centre of a 1-inch copper tube bent to radius and extending two-thirds round the circumference of the vat. The coil is perforated with eighty jets $\frac{1}{16}$ inch in diameter on the inside of the curve. The coil is plugged at both ends with a taper screw plug and clipped 3 inches from the bottom and side of the vat by means of brass brackets and screws.

The 2-inch cold-water service terminates in a gate valve at the top of the vat and a 1-inch hose cock is also placed so that it may be operated from the inside of the vat during cleaning operations. The 3-inch flanged plug cock and discharge pipe are bolted to the fall side of the vat bottom in a position most convenient for their opening and closing from the mash tun staging below.

The Mash Tun. The capacity of this vessel depends upon the output of yeast required, and in this instance it is calculated to the dimensions necessary to mash 27 cwt. (1,371 kg.) of mixed raw materials, but both the starch cooker and mash

tun are slightly oversize for mashing with the normal ratio of $3\frac{1}{2}$ by weight of water to 1 of raw material; the extra space being provided in the starch cooker for the froth development when crushed wheat is boiled, and in the mash tun to allow for possible inclusion of a higher percentage of malt culms in the mash than that usually required.

When milled raw grain or malt is added to water the volume of the resulting mixture is considerably greater than the original volume of water; there is only a slight difference in the total volume when different cereals are mashed separately, but for practical purposes the increase in volume may be taken as a constant, for instance, 1,120 lb. of barley malt mixed with 336 gallons of water increased the volume by 87 gallons; the same amount of crushed raw barley shows a difference of 89 gallons, and wheat and tapioca both increased the volume by 87 gallons. Dry malt culms absorb more than three times their weight of water and thus swell considerably in the mash tun, but their expansion varies widely with their quality.

In practice it will be found that the weight of the raw material in the differential grain brew described will occupy a volume equivalent to the same weight of water.

The mash tun (see Fig. 16) is 8 feet 6 inches in diameter and 5 feet deep, allowing 9 inches freeboard above the level of the mash. The staves are 67 inches long, 4 inches wide backed and hollowed from $3\frac{1}{2}$-inch timber with a $4\frac{1}{2}$-inch chimb and a $2\frac{1}{2}$-inch croze $\frac{3}{4}$ inch deep. The hoops are made from 1-inch iron rod bent to radius and bolted through 9-inch cast-iron shoes. The bottom is made of 3-inch timber dowelled and chamfered similar to that of the starch cooker.

The mountings and fittings necessary on the mash tun are similar to those on the starch cooker except that a cooling coil is added and there are differences in measurement due to the difference in size of the vessels. The tun is furnished with counterpoised sheet metal covers and agitators, cooling coils, steam coils, hot- and cold-water service and a discharge cock and tubing.

The agitator is of the propeller type, with blades 7 feet 6 inches overall length, rotating at a peripheral velocity of 700 feet per

minute, and driven from the top of the tun by an 18-inch crown wheel and 6-inch pinion; the ratio of these mitre gears

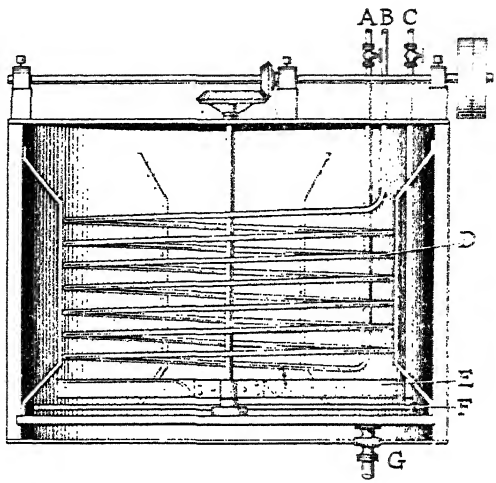


FIG. 16. Mash tun (in section).

A. Cold water inlet to coils B. Discharge from cooling coil. C. Steam inlet. D. Cooling coil. E. Agitator. F. Steam coil. G. Discharge cock.

is usually 3 to 1, but it may be an advantage to reduce the ratio slightly and vary the meshing by cutting twenty-one teeth on the pinion and sixty-four teeth on the crown wheel.

The pinion shaft is bracketed to the channel iron gear support and fitted with 24-inch fast-and-loose pulleys with 4-inch crowned faces.

The length of tubing required for the cooling coil is estimated by allowing 1 square foot of cooling surface for every 45 gallons of the vessel's mash capacity, but the most efficient tubing has a diameter that, in relation to the size of the vessel, offers the least resistance to mash agitation and yet retains the optimum temperature during the mashing process without extending the time.

From the particulars given the dimensions of this vat require 40 square feet of cooling surface and by using $1\frac{1}{4}$-inch diameter tubing it is found that 125 lineal feet of copper pipe are necessary. The tubing is bent to form coils with a

diameter of 6 feet 6 inches, spaced with gunmetal clips at 6-inch centres on $2\frac{1}{2} \times \frac{1}{4}$ inch flat copper standards that are bent to shape and bolted top and bottom to the mash tun staves with brass bolts, the lowest coil clearing the agitator blades by 3 inches.

Tested brass unions are used to join the length of tube in assembling the coil, and the outlet and inlet are taken through the channel steel support. The flow of water or cooling medium is regulated by a stop cock placed conveniently near the mash tun control platform.

The cold-water supply and steam service fittings are similar to those of the starch cooker, the hot-water service is supplied from the hot-water vat through a 2-inch supply pipe; this vessel is installed to economize in fuel by using the hot waste water from the wort cooler and from the exhaust steam heaters.

The mash discharge cock is placed as close to the bottom of the fall side of the tun as possible, and to ensure that the grains cannot choke the cock by packing in a length of tubing during mashing operations a 3-inch flanged plug cock is bolted directly on to the bottom of the tun and a 3-inch discharge pipe conveys the mash to the centre of the filter tank below.

The Filter Tank (Fig. 17). This vessel is used to filter the wort and to wash residual extract from the grains. The tank is usually made in the form of a shallow rectangular vessel,

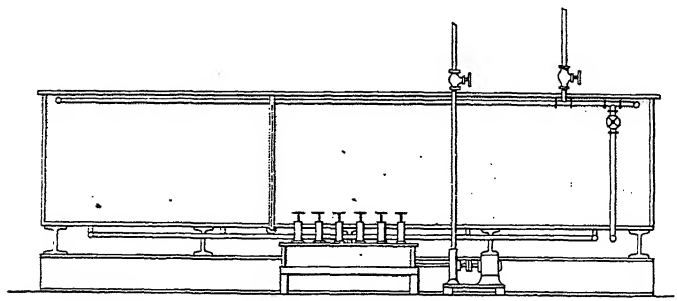


FIG. 17. Diagrammatic sketch of filter tank.

but its actual shape is immaterial provided that sufficient filter area is allowed so that the wort may be completely

extracted from the grains. As a rule 3·5 square feet of filter surface are allowed for each hundredweight of raw material used in the mash. This figure is regarded as a guide only because filtration efficiency is not a function of filter area alone but depends almost entirely upon the condition of the grains and the complete conversion of the starches.

It is possible to filter wort through double the depth of grains that the above ratio of filter area to grain allows. This practice is not economical unless means are provided for a periodical agitation during wort filtration in order to break up the cracks and channels that form and then allow the sparge water to flow through to the filter plates without washing any of the extract from the grains.

In this instance the tank is 10 feet square and 3 feet deep. It is constructed of boiler plate and fitted with sparge pipes and underlet, a false bottom of slotted brass or perforated copper filter plates, a system of drainage pipes for the filtered wort, filter gauge and a manhole with cover for spent grain discharge.

The bottom of the tank is made of $\frac{1}{4}$-inch boiler plate with electrically welded joints ground to a smooth finish. The angle between the bottom and the sides is rounded by bending 2 inches of the outside edge of the bottom plate upright, and when this is caulked it spreads to form a ledge that supports the extreme edge of the false bottom.

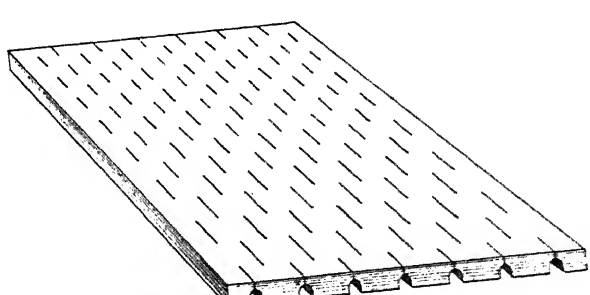


FIG. 18. Brass filter plate.

The sides of the tank are made of $\frac{3}{16}$-inch boiler plate and riveted to the outside of the turned-up portion of the bottom.

The top edge of the tank is finished with a stiffening rim of 2-inch angle iron riveted internally and thus forms a protecting ledge under which the sparge arms are clipped.

The filter plates forming the false bottom (Fig. 18) may be cut from brass plate and slotted as shown; but the initial cost is considerably reduced without loss of efficiency if they are made of heavy copper plate, each one of which is cut to measure 5 feet long and 2 feet 6 inches wide with an underlapping strap 1 inch wide covering the joints between each plate.

With the exception of an area blanked by these straps the plates are perforated by punching $\frac{3}{32}$-inch holes at $\frac{1}{2}$-inch pitch and they are supported 2 inches off the true bottom of the tank by three rows of brass studs burred and sweated into each plate at 8 inches pitch.

The filtered wort is drawn from the tank through four horizontal lines of 1-inch copper tube spaced 24 inches from centres, running from back to front and 2 inches under the bottom. Each tube is connected to four escape holes drilled in the tank bottom, at 24-inch pitch, by brass flanges and tee pieces. The pipes project 4 inches in front of the tank where tubing and tee fittings are used to group the ends at the pump suction box; a gooseneck fitting and milk tap is then attached to this end of each tube to raise the level of its discharge to the level of the false bottom (see Fig. 19).

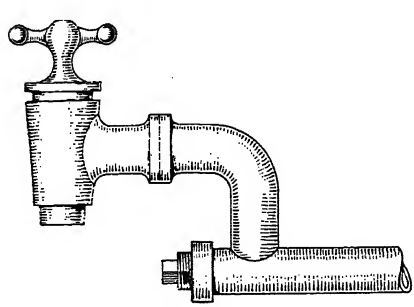


Fig. 19. Milk tap and gooseneck.

The pump suction box, or "grant," is a rectangular copper vessel 24 inches long, 12 inches wide and 10 inches deep, and is placed beneath the wort taps in front of the filter tank to receive the filtered wort. The grant is connected to the wort pump with a short length of copper tubing and is furnished with a screw plug in the bottom for flushing purposes. It is covered with a 50-mesh wire sieve to retain any particles of

grain that may be drawn from the filter tank with the wort; this strainer is sweated to a brass frame that allows the sieve to drop loosely 1 inch inside the grant so that it may be removed and cleaned when necessary.

In an effort to reduce infection risks a more elaborate attachment is sometimes installed instead of the grant. This refinement consists of a horizontal copper cylinder 10 inches in diameter and about 30 inches long enclosing a cylindrical sieve of slightly smaller diameter. The sieve is attached to one of the two head plates that close the ends of the cylinder by means of rubber gaskets and wing nuts. The wort discharges from the taps through cylindrical sight glasses into the cylinder and then to the pump without exposure to the atmosphere. Theoretically the apparatus should afford greater protection from air-borne infection, but in practice this advantage disappears if, during wort filtration, the garniture requires frequent opening to clean the sieve.

The flow of sparge liquor is controlled by a gate valve set on the 2-inch hot liquor main in front of the tank, and it is distributed from each end by a sparge pipe that enters at the top of the tank and is clipped along the inside edge under the angle iron rim. These sparge pipes are 1-inch copper tubes drilled with three rows of fine holes that are set to distribute jets of hot sparge liquor evenly and lightly over the surface of the grains (see Fig. 20).

The underlet is an extension from the hot liquor main controlled by a valve placed below the sparge connection. Its function is to discharge hot water between the filter plates and the bottom of the tank for the purpose of lifting the grains off the filter plates and filling the tank rapidly with hot water when it is found necessary to agitate the grains to secure their complete extraction.

The manhole is 12 inches in diameter and situated in the most convenient position for the spent grain discharge. If this is near a corner of the filter tank the brass manhole cover may be operated from inside the tank and seated firmly on a rubber gasket by means of a long screwed rod attached to the cover and threaded through a bracket placed across the corner at the top of the tank. Otherwise a hinged manhole

cover is operated from beneath the tank and firmly seated with tightening bows fastened beneath the tank bottom (see Fig. 21).

The Wort Cooler. The method generally adopted in wort refrigeration is known as the counter current principle in which the cooling medium flows in a direction opposite to the

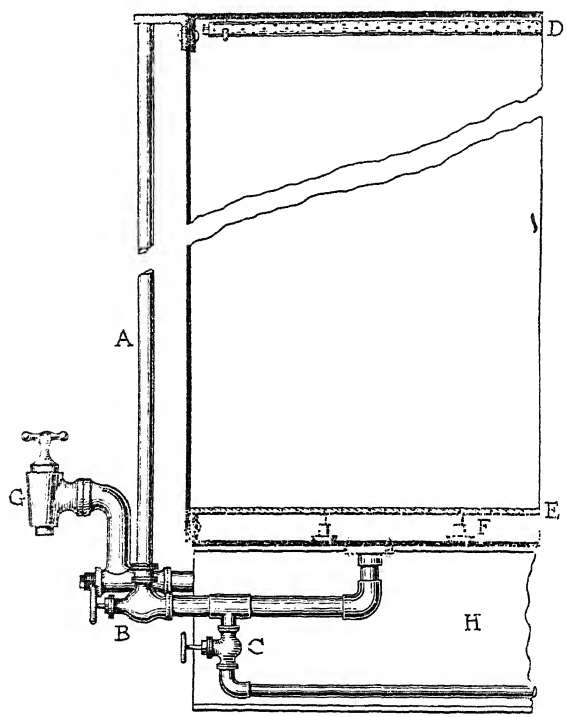


FIG. 20. Section of filter tank showing filter gauge and other fittings.

A. Gauge glass. B. Angle valve. C. Steam valve. D. Fixed sparge pipe. E. Perforated false bottom. F. Studs supporting false bottom. G. Wort drainage tap. H. R.S.J. tank support.

flow of wort, thus the cold water or brine enters the cooling system at the bottom where it encounters the lowest temperature of the wort and discharges at the top where it becomes nearly as hot as the wort is at that point. Cooling efficiency is influenced by a number of factors which includes the velocity with which the wort flows over the cooler, the thickness of

metal separating the wort and cooling medium, and the range of temperature reduction desired.

In distillery practice, or when producing yeast from spirit brews, wort cooling is simplified because the reduction in temperature necessary to commence fermentation is within the range where service water alone may be used as the cooling medium and a simple horizontal refrigerator may be employed.

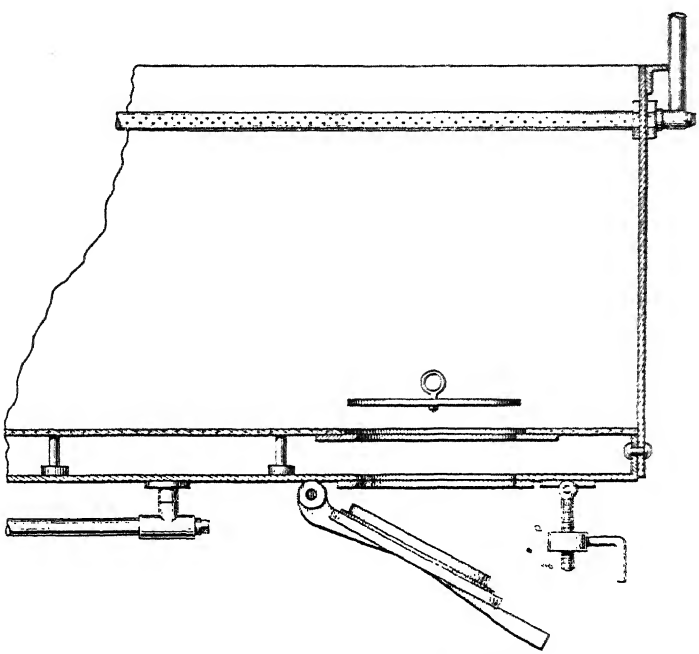


FIG. 21. Section of filter tank showing a method of closing the manhole.

This type of cooler occupies very little space, and is eminently suited for buildings of restricted height. The wort flows through a series of tubes around which the cold water circulates in a shallow box like housing, and it has another valuable feature in that the hot wort flows from the filter tanks to the refrigerator, where it is chilled, and gravitates to the fermenter without exposure to air-borne infection.

The principle of operation and the construction of horizontal

refrigerators vary slightly in different makes, but their cooling effect is very much the same and in general their efficiency ranks about 25% below that of the vertical coolers.

In selecting a refrigerator an essential feature is the ease and certainty with which the unit may be cleaned and sterilized; all surfaces must be perfectly smooth and castings free from blow holes. This applies particularly to those fitted with tube plates, which should be finished without projections or crevices of any description.

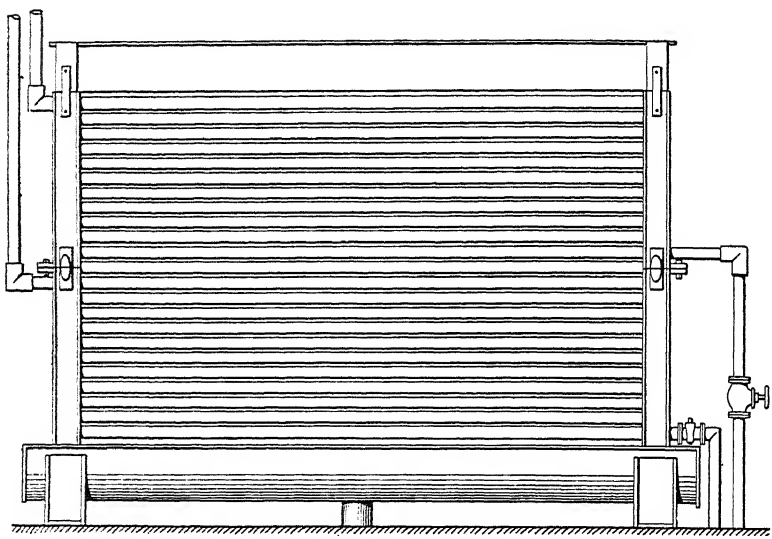


FIG. 22. Baudelot cooler.

The vertical cooler (Fig. 22) is constructed upon an entirely different principle. It consists of a series of horizontal tubes arranged in a vertical tier about 7 feet high. The tubes are soldered into upright standards, or headers, of cast brass moulded so that the cooling medium flows from the bottom through each tube in turn until it is discharged from the top tube of the series.

The cooler is made in two sections so that service water only flows through the upper section, whilst either chilled or service water may flow through the lower section as required, by means of stop cocks and fittings on the water service pipes.

The wort from the filter tank is discharged into a vee-shaped trough placed at the top of the cooler which distributes it over the full length of the cooler by means of small perforations in the bottom of the trough. A narrow strip of sheet copper with its lower edge shaped like saw teeth, and known as the drip strip, is soldered along the bottom of each tube to direct the flow of wort from one tube to the other without splashing. The wort descends in a thin film over the tubes and falls into a collecting pan about 9 inches deep, in which the refrigerator stands, fitted with an escape tube and connections that conduct the wort into the store tank or fermenter as desired.

During the cooling process the wort is continually exposed to the atmosphere, for a period extending from two to four hours, in a thin film covering the full area of the cooler, and infection is inevitable unless preventive measures are employed; the refrigerator is therefore enclosed in a chamber to which air is admitted only through a ring air filter, and this installation provides an opportunity to increase the efficiency of refrigeration by the added effect of evaporation in a forced draught. A current of filtered air that enters the chamber at floor level, becomes moisture laden as it rises around and over the refrigerator and is exhausted with the vapours from the hot wort by means of a multivane fan placed in the roof vent above the cooler.

Vertical coolers of the dimensions shown below are necessary to chill wort from 160° F. to 60° F. when service water at 65° F. and chilled water at 40° F. are used as cooling media.

Number of Tubes.	Length of Tubes.	Diameter of Tubes.	Approximate Height of Cooler.	Gallons of Wort per Hour.
22	7 ft. 0 in.	2 in.	4 ft. 11 in.	250
24	7 ,, 6 ,,	2 ,,	5 ,, 4 ,,	300
26	8 ,, 0 ,,	2 ,,	5 ,, 9 ,,	400
28	9 ,, 0 ,,	2 ,,	6 ,, 2 ,,	525
30	10 ,, 0 ,,	2 ,,	6 ,, 8 ,,	650
32	12 ,, 0 ,,	2¼ ,,	7 ,, 1 ,,	850
34	12 ,, 0 ,,	2¼ ,,	7 ,, 6 ,,	1,100

Wort Store Tank. So much depends upon the sterility of the flow of strong wort in a differential brew that the success

cover is operated from beneath the tank and firmly seated with tightening bows fastened beneath the tank bottom (see Fig. 21).

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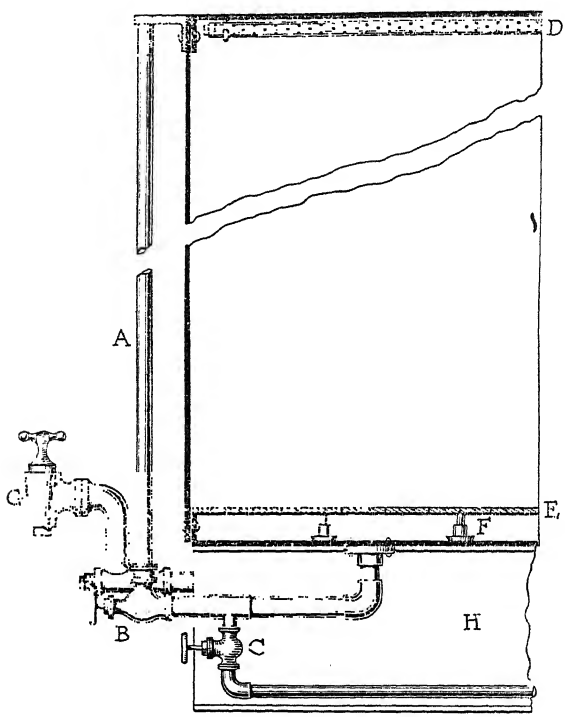

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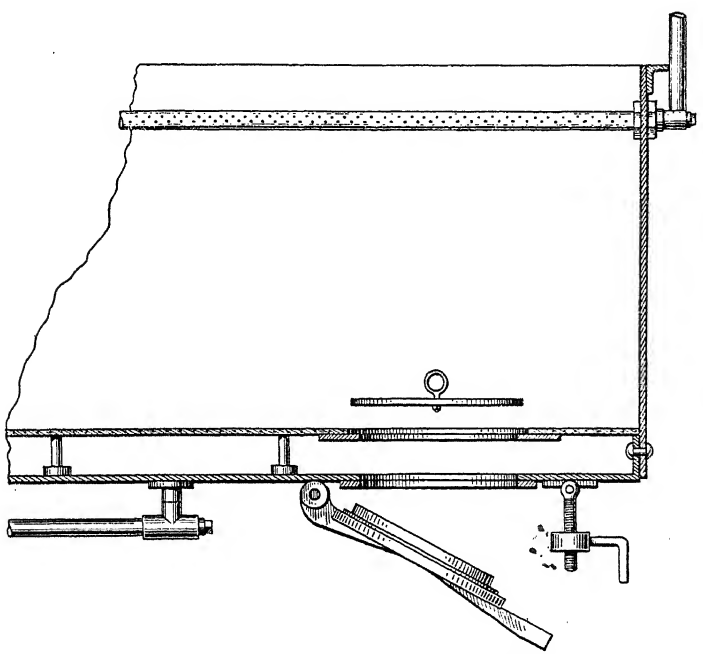

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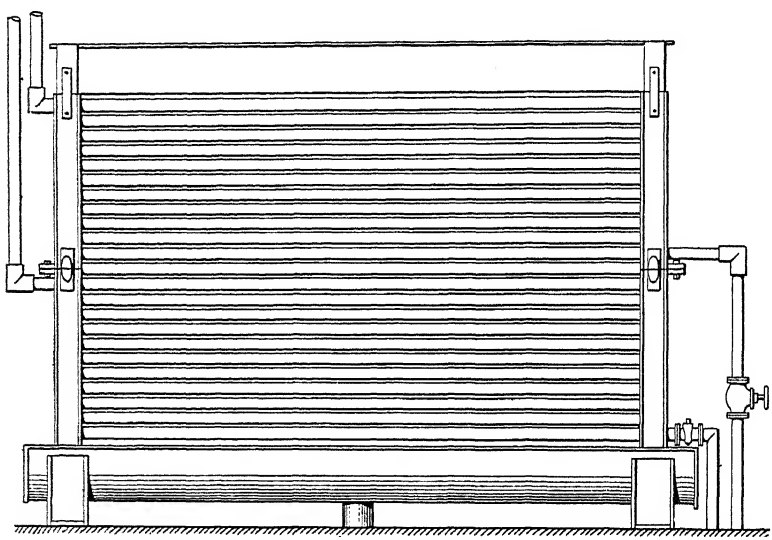

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32	12 ,, 0 ,,	2¼ ,,	7 ,, 1 ,,	850
34	12 ,, 0 ,,	2¼ ,,	7 ,, 6 ,,	1,100

Wort Store Tank. So much depends upon the sterility of the flow of strong wort in a differential brew that the success

of the fermentation rests to a considerable extent upon the design, construction and maintenance of the wort store tank.

The strong wort and first spargings from the grains are mixed and stored in this vessel for about fifteen hours, or from the commencement of wort filtration until the last of the stored wort flows into the fermenter, and to keep this volume of wort sterile for such a long period requires careful supervision. This effort may be wasted and the yeast infected by the use of a faulty or unsuitable wort storage vessel.

The tank is 108 inches in diameter, 72 inches deep and made of light sheet metal. All joints are smooth finished and corners are avoided by rounding the angle, where the sides meet the bottom, to a radius of not less than 4 inches.

The top edge is stiffened with an external ring of 2-inch angle iron to which the cover is riveted and caulked tight on the inside. The top of the tank is furnished with a manhole and cover, an air pipe for wort agitation, a steam pipe for sterilization, a wort level indicator and a wort delivery tube from the refrigerator. The bottom of the tank is furnished with a wort discharge regulating valve and tubing that delivers the wort to the atomizing sprays. The outside of the tank is completely covered with 3 inches of mixed fireclay and asbestos insulation, trowelled to a smooth finish to prevent the absorption of heat by chilled wort, or the radiation of heat by hot wort.

The 18-inch manhole is edged with an angle iron ring welded to the top of the tank and closed with a manhole cover; an airtight joint is made by tightening the edge of the cover on a gasket with wing nuts. The tube that delivers the wort from the refrigerator, or filter tank, enters the tank through a large diameter flange sweated to the cover, and dust is excluded by means of a cap attached to the wort pipe to form an annular cavity that fits over the tubular portion of the flange.

A wort level indicator is essential in controlling the flow of store wort, and of the two kinds in use the electric type is preferred for its accuracy and cleanliness. The mechanical level indicator consists of a flat-bottomed ball that floats on the wort and is attached to a length of jack chain passing up and through the top of the tank, over a small pulley hooded

to exclude dust, and down in front of a graduated indicator board where it is held taut by a plummet. This type is a continual source of danger owing to the possibility of infection entering the vessel with the chain.

The capacity of the tank and the method of graduating the indicator board to show the volume of wort that is delivered each hour is described in the differential fermentation of an all-grain brew.

A 2-inch branch pipe from the air main is connected to the vessel by a copper pipe that pierces the cover and descends within 2 inches of the bottom of the tank where a 6-inch length is bent at right angles. This pipe is a permanent fixture, fastened with back nuts and sweated to the cover. A 2-inch gate valve controls the admission of air that is used to agitate the store wort only when it is sampled before the differential flow commences ; a $\frac{3}{4}$-inch steam pipe is connected to the air line between the air valve and the tank and effectively sterilizes this tube and the tank at the same time.

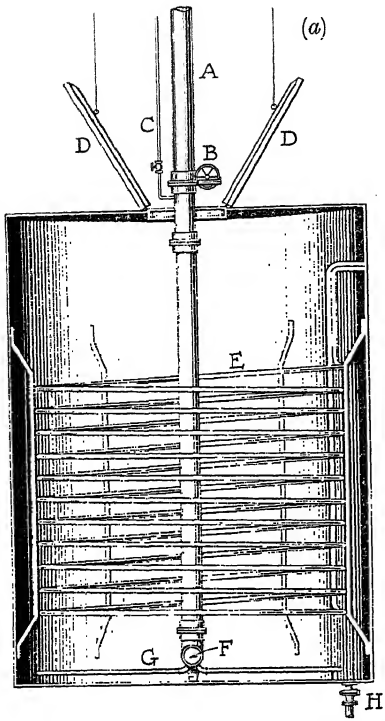
The wort is discharged through a flanged plug cock riveted on the bottom of the fall side of the tank and is connected by a sanitary union with a straight length of copper tube, which terminates at the top of the fermenter with a plug cock fitted with a pointer that indicates the volume of wort discharged through the atomizing sprays on a graduated scale.

Seed-Yeast Tank. In all differential processes of fermentation the use of any unit or attachment in excess of the essential equipment is simply regarded as another potential source of infection, and for that reason the installation of a seed-yeast tank is inadvisable ; but in the spirit fermentation of worts it is sometimes necessary to place the wort under the protection of actively fermenting yeast as quickly as possible.

When this procedure is adopted the seed-yeast tank is charged with strong wort, kept in a sterile condition from a previous brew, and inoculated with seed yeast several hours before the filtration of wort from the mash commences.

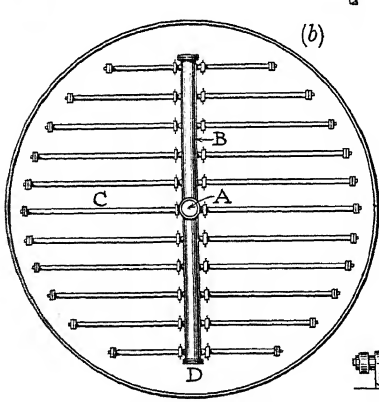
Fermenter (see Fig. 23). The most convenient situation for the fermenter is on the first floor of the building and process control is simplified if space allows the filter tanks and laboratory to be installed on the same level.

FIG. 23. Fermenter.



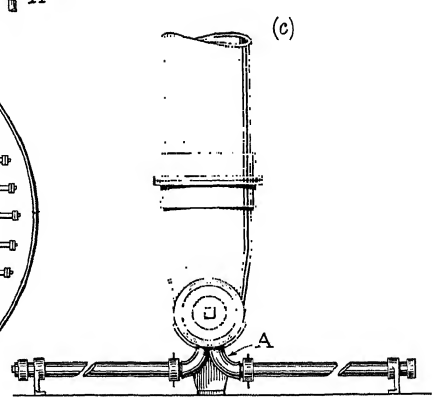
A sectional view of the fermenter showing the essential fittings.

A. Air main.
B. Blast gate.
C. Steam pipe.
D. Counterpoised covers.
E. Cooling coils.
F. Horizontal air main.
G. Air distributing pipes.
H. Discharge cock.



Plan of the air distributing pipes at the bottom of the fermenter.

A. Vertical air main.
B. Horizontal air main.
C. Distributing pipes.
D. Screw caps.



The improved method of attaching the air distributing pipes to the horizontal air main in the fermenter. The curved fitting (A) facilitates cleaning operations, and automatically drains the air main; essential features of the system includes rapid assembly, tight joints, and the correct direction of air jets.

The fermenter may be constructed of either copper, stainless steel, heavy gauge sheet metal, or boiler plate. Wood stave vessels have been used as temporary expedients only, but their heavy maintenance charges equal those of the periodical scaling and varnishing necessary to keep a boiler plate fermenter in good condition.

The fermenter is subjected to exceptionally severe conditions daily and it can give satisfactory service only when it is soundly constructed, rigid in shape, and firmly supported. It contains a live load weighing up to 20 tons of turbulent wort, agitated, in the example brew, by the injection under pressure of more than its own volume of air every minute during fermentation, and the metal and its fabrication is further tested by the wide range of temperatures employed in sterilization and fermentation.

In the differential fermentation of a brew of 1,371 kg. (27 cwt.) of raw material diluted with fourteen parts of water to one of raw material, 19,194 litres of wort are produced, and at a depth of 3 metres this equals the volume of a circular tank with a diameter of 289·5 cm. (114 inches), but owing to the turbulency of the fermenting liquid under intense aeration ample freeboard above the nominal surface of the liquid is necessary to avoid overflowing, and this is provided by designing the tank with a total depth of 457·2 cm. (180 inches).

The sides and bottom of the vessel are made of stainless steel, all joints are either strapped outside and secured with countersunk rivets or the joints are electrically welded inside and ground to a smooth finish. The angle where sides and bottom meet is rounded to a 3-inch radius and is strengthened at the joint with an external ring of 2½-inch angle iron. The sides are braced with six tee iron uprights welded to the bottom ring and to a similar angle iron ring attached to the top edge of the tank to give it rigidity.

The vessel is furnished with sheet metal covers, an air-distributing system, steam service, cooling coils, hot- and cold-water services, recording potentiometer and thermometer, discharge cock and connections, and a small steam-heated fat pot.

The covers are counterpoised and made in two segments of

light sheet metal on an angle iron frame; each cover is hinged to a length of 4-inch angle iron which spans the tank and supports the air main between them, the intervening space being covered with a fixed strip of sheet metal.

The amount of tubing required in the cooling coil is estimated by allowing 3·5 square feet of cooling area for each hundredweight of raw material brewed; thus the temperature of fermenting wort from 27 cwt. of raw material is controlled with 94·5 square feet area. In addition to the temperature and interval of contact, or velocity of the cooling medium through the coils, the diameter of the tubing is a contributing factor in cooling efficiency and 1½-inch tubing is generally employed, therefore 240 feet of copper pipe is necessary plus the length required to carry the inlet and outlet of the coil to the top of the tank.

The lengths of tubing are bent to form a coil with a diameter of 8 feet, joined with tested unions and spaced 6 inches between centres on six flat 2-inch copper standards attached to the sides of the tank. The coils are clipped in position with hooks of ½-inch brass rod threaded one end to bolt at the back of the standard.

The valve controlling the flow of cooling medium is situated on the inlet pipe which descends from the top of the tank to the first coil, placed 12 inches from the bottom, and at its lowest point is furnished with a 1-inch plug cock fitted with a locking device to prevent leakage during fermentation. This cock and one similar at the top of the outlet pipe are required to drain the cooling coil before the tank is steam-sterilized.

Air is conducted from the compressor to the fermenter through a vertical sheet metal air main, 7½ inches in diameter, that enters the tank through the centre of the cover and connects with a 5-inch horizontal main supported on studs 3 inches from the tank bottom.

The air is injected into the wort through three rows of $\frac{1}{16}$-inch jets drilled along the underside of twenty-four transverse distributing tubes, 1½ inches in diameter, attached at right angles to the horizontal main on the bottom of the tank. An even distribution of air is secured by maintaining a slightly higher pressure inside the tubes than at the jets and, for this

reason, the aggregate area of the jets should not exceed 90% of the cross section area of the parent main. The pitch of the jets is then determined by the number that are to be drilled at equal distances in the total length of tubing.

These horizontal air-distributing tubes are furnished with unions at one end and, in opposite pairs, are screwed on to twelve moulded fittings riveted and sweated at equal distances along the underside of the horizontal main. The free end of each tube is supported in a perfectly horizontal position by an adjustable stud secured with a locknut on the plug that closes the end of the tube.

The volume of air that flows through the system is controlled by a blast gate on the vertical main ; this is supported above the cover by the angle iron cross members, and is operated from the side of the tank by a long extension of the blast gate pinion spindle. The volume of air injected into the wort is indicated on the chart of a recording air volume meter, the recording pen of which oscillates according to the differences between the static and dynamic pressures developed by the velocity of the air flow as it passes through a pitot flange, or over pitot tubes, installed in the suction duct of the air compressor.

In the absence of an air volume meter the periodical variations in the speed of the compressor necessary to deliver the volumes of air required during fermentation may be determined by the installation of a differential manometer.

A $\frac{3}{4}$-inch steam service is connected to the air main below the blast gate for the purpose of sterilizing both the air-distributing system and the fermenter immediately before fermentation commences, and a hot- and cold-water service terminates at the top of the tank with hose cocks and sufficient hose to reach the most distant part of the tank.

The fermented wort is discharged, and flows to the separator feed main, through a 3-inch flanged plug cock attached to the lowest part of the tank bottom and then through copper tubing joined with sanitary unions that facilitate cleaning. In many cases the discharge cock escapes much of the heat of sterilization, and it should be constructed and placed in such a position that it may be readily dismantled and sterilized before each brew.

The Air Compressor. This unit is one of the most important in the yeast factory and should receive the greatest care in selection and maintenance. The pressure developed against the compressor in 118 inches (3 metres) of wort amounts to nearly 4·5 lb. per square inch, and this with the wide range of volumes required each day restricts the choice of a machine to the very few that can give unfailing and economical service under most exacting conditions.

The multistage centrifugal compressor represents the latest development, combining economy, flexibility and silence in operation with so many other desirable features that this type is ideal for the modern yeast factory.

It will maintain practically a constant pressure over a wide range of volumes and, as the volume of air required decreases, the power required by the compressor will also decrease although the pressure will remain nearly constant. The machine is therefore self governing in operation both as to quantity of air handled and power consumption.

The pressures developed cannot rise above a predetermined maximum which is only slightly higher than the pressure for which the unit is designed. This feature, together with that of self governing, eliminates the necessity for relief valves and by-pass connections and the possibility of damage when either the supply or discharge line of the compressor is closed completely.

This point is important and will be appreciated for its obvious advantages and economy in the yeast factory where the volumes of air required vary widely according to the yeast generation, the type of brew and during the fermentation of each brew.

In the multistage centrifugal compressor there is no contact between stationary and moving parts except in the bearings. The absence of contact and friction within the compressor insures constant performance and efficiency regardless of how long the apparatus is in operation. The stationary casings are made of welded steel and the design of the unit enables direct connection to motors or turbines. This not only eliminates all belting and gearing, but makes it possible to furnish a self-contained unit consisting of compressor and driver mounted on

a common base, occupying less floor space than any other type of apparatus performing the same duty.

Air Filter. An adequate supply of sterile dry air is essential to the process but elaborate air conditioning equipment is quite unnecessary; the absolute purity of the air supplied to the fermentation is secured by simple filtration through a suitable medium.

The most efficient air filter yet developed for this purpose consists of a filter bed of glycerine-coated sheet metal rings, and filtration is effected by the numerous changes in direction of the high velocity air current as it passes through the rings. Dust particles in suspension cannot change their direction of flight with the same rapidity as the air current and consequently impinge upon the moist surfaces of the rings where they are retained by the glycerine, and this becomes heavily charged with dust particles and spores.

Purified glycerine is periodically sprayed on the top surface of the ring filter and this carries the impurities into a draining

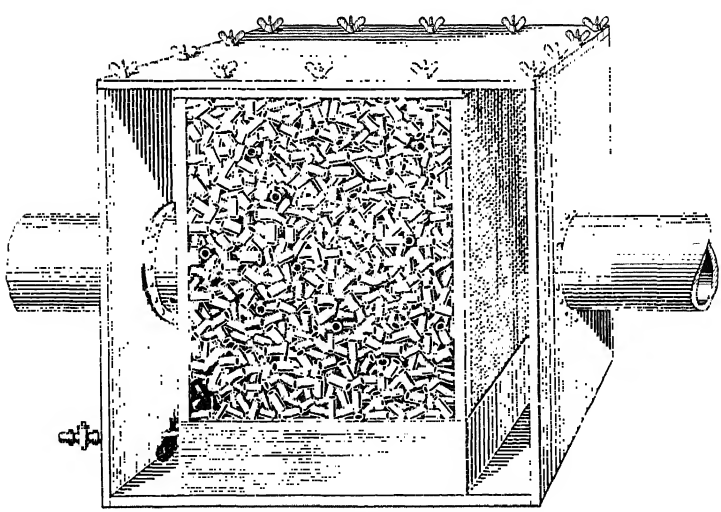


FIG. 24. Section of ring-type air filter.

tray from which it is removed, heated to destroy infection and filtered bright before it is returned and sprayed on the filter rings.

This type of air filter, shown in Fig. 24, offers no resistance to the air current and consists of a 36-inch square wire basket with a sheet metal tray, 3 inches deep, to the bottom of which an 18-inch length of ½-inch tubing is attached and closed on the outside of the filter housing with a plug cock. The wire basket is completely filled, in disorder, with light sheet metal rings 1 inch in diameter and 1 inch long, made by winding 1-inch strips of sheet metal round a mandrel.

The filter basket is totally enclosed in an air-tight, close-fitting, felt-lined wooden casing with a clear space of 12 inches between the wire basket and the blower suction duct, to which the filter housing is attached with an air-tight joint; the same distance separates the filter from the front of the casing which admits the impure air to the filter basket through a 12-inch orifice.

The top of the housing is secured with wing nuts, and with the bottom and sides fit close to the filter basket so that the air current in passing from the front to the back of the housing must pass through the filter rings.

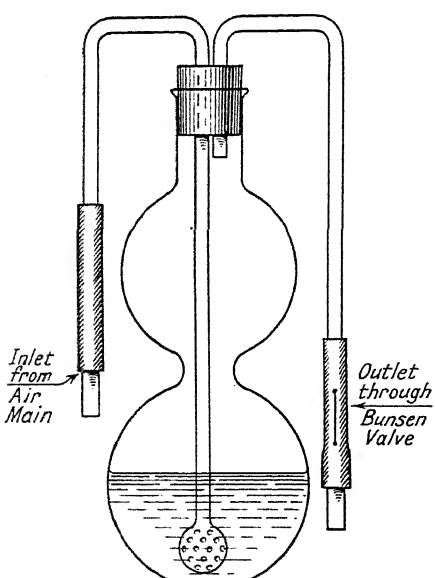


Fig. 25. Aeroscope.

The Aeroscope (see Fig. 25). The purity of the air supply is tested at intervals by means of an aeroscope containing 100 c.c. of sterile wort. An essential feature of this apparatus is the glass aeroscope tube with a round bulb on one end with about twenty small perforations through which the air passes at a rapid rate but is so finely divided that every particle is brought in close contact with the sterile wort.

This tube is fitted into a 500-c.c. flask, a portion of the neck of which is expanded to form another bulb ; by means of a double perforated stopper, which also bears a short bent tube, the exposed end of which is closed with a Bunsen valve, the upper end of the aeroscope tube passes through the stopper and is bent down to prevent infection and dust falling into the open end of the tube.

The apparatus is connected to a pett cock on the air main with a piece of sterile rubber tubing and about 2 litres of air is passed through the wort in a gentle current. The aeroscope is incubated at 86° F. for forty-eight hours, then microscopically examined, and if any micro-organisms develop in the wort the air suction duct is steam sterilized with formalin, the air filter is opened, the rings washed and sterilized with formalin, dried, replaced in the filter basket and saturated with purified glycerine.

Yeast Separators. Yeast was formerly separated from the wort in which it was grown by a settling process in specially constructed shallow tanks and its sedimentation occupied a period of from five to fourteen hours. This interval was considerably reduced by the development of processes promoting the growth of flocculent yeast which effected a speedier clarification of the wort, but the result of keeping yeast at comparatively high temperature in contact with alcoholic wash, even for the shorter periods of sedimentation, was often disastrous to its baking quality and led to experiments that resulted in the mechanical separation of yeast and wort.

Marked improvement in yeast quality was observed following the introduction of centrifugal yeast separators. In operation separators rely upon the effect of centrifugal force upon a mixture of liquids and solids of different densities ; the yeast is heavier than the wort in which it is suspended, and when this mixture is rapidly rotated in a confined space the yeast is thrown to the outer periphery whilst the wort is found quite clear in the centre. Thus the principle underlying the centrifugal separation of yeast and wort is quite simple but the application of this principle is quite the reverse ; the difference in specific gravity between yeast and wort is so slight that very high speeds are necessary and in consequence the separators

must be designed with precision and assembled in perfect balance. Skill and care is also necessary in their operation and maintenance.

Changes in the yeast industry led to the evolution and final perfection of non-alcoholic fermentation processes ; and continual improvement in separator design and construction was necessary in order to meet the changing demands for greater separator capacities with worts of widely varying yeast concentrations. In 1926 the Westfalia Separator Co. produced machines with a capacity of 5,000 litres per hour, and before 1928 this output was further increased to 10,000 litres per hour.

The advantage that would follow the perfection of an electric-driven machine was appreciated by separator engineers and was the subject of research for many years. Attempts were made to drive separators with a motor arranged vertically on the bowl spindle, but the matter was not as simple as it appeared; and the belt and troublesome steam turbine-driven machines were in general use until the problem was approached from a different standpoint and the modern highly efficient direct motor-driven separators were produced.

Before non-alcoholic processes of yeast culture were perfected the fermented wort contained from 2% to 2·5% yeast and the nominal capacity of separators was calculated on that basis ; but with the perfection of new yeast cultivation processes the yeast content in some worts increased to 14% or even higher in those processes where excessive amounts of seed yeast are employed. This change naturally influenced the capacity of separators designed to separate worts with the lower yeast content and demonstrated that the basis upon which separator capacities was calculated required revision and a modification in machine design. After further research the Westfalia high-capacity separator was produced in 1930 with a nominal capacity of 20,000 litres per hour at 5,000 r.p.m.

This model is remarkably flexible in its operation and consistently produces a yeast cream containing 60% yeast from a wide variety of fermented worts with yeast concentrations varying from 1·5% to 14·5% ; and this high order of efficiency is maintained at speeds varying from 4,500 to 5,500 r.p.m.

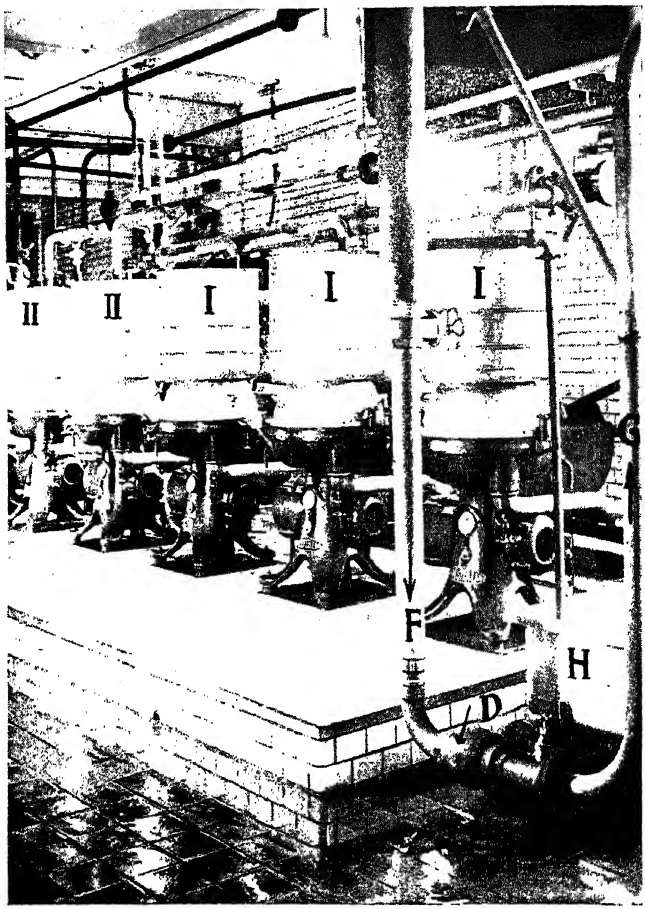


FIG. 26.

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A distinct advance in the finishing processes of yeast manufacture was effected in 1933 by the introduction of the Westfalia yeast washing nozzles, by means of which the yeast concentrate, from molasses worts, is washed, chilled and separated in one operation. The durability of the yeast thus treated is increased by reducing the interval of time between the end of fermentation and the cold storage of the filter-pressed yeast. Time and labour is saved and the space formerly occupied by yeast-washing tanks, pumps and pipelines may be used for other purposes.

In this system the yeast concentrate from the wort separators is drawn into a specially designed injector where it mixes with chilled wash water under a pressure head of 6 metres, or sufficient to elevate the mixture of yeast and wash water to the inlet receiver of the washing separators; the chilled concentrate is then filter-pressed in the usual manner.

The direction of flow of concentrate is shown in Fig. 26. The yeast concentrate from the wort separators (I) flows into the small receiver (H), from which it is drawn by the injector (D), mixed with wash water from the pipeline (F) and delivered through the line (G) to the wash separators (II).

Yeast Cream Tank. This vessel is installed to hold the separated yeast concentrate that accumulates during the opening and closing of the filter-press and to act as a reservoir supplying the filter-press pump. It has a capacity of about 600 gallons and is furnished with a mechanical agitator, an air distributing system, and cooling coils. The latter is connected to the chilled brine service, and when an appreciable volume of yeast concentrate accumulates in the tank the brine circulating through the yeast cream cooler is returned through the coils in this tank before it returns to the brine tank for refrigeration.

The tank is connected to the suction of the filter-press force pump by a 2-inch copper tube fitted with sanitary unions and a plug cock with a by-pass to the drain for cleaning discharge. Between the plug cock and the pump a reducing tee is fitted to the suction tube into which is screwed a 36-inch vertical

length of 1-inch copper tube closed with a pett cock. This tube admits air to the pump when it is required to blow out the residual liquid in the filter-press, and in drying out the cakes of yeast before their discharge.

The Filter-press and its Operation. Yeast is discharged from the separators in the form of a cream, or concentrate, in which it is present in the proportion of one part of yeast to four parts of spent wort.

In order to recover the yeast in the condition in which it is distributed to consumers the yeast cream is filtered through cloths supported on the plates of a filter-press, and from this operation it takes the name of compressed yeast which distinguishes it from brewer's liquid yeast, and the concoction known as spontaneous yeast prepared by individual bakers.

Owing to its perishable nature, yeast rapidly deteriorates at room temperature when deprived of spent wort, and its rapid filtration is imperative ; but the delay and difficulty that so often attended this process has been eliminated by the improvements in filtration technique and filter-press design introduced by S. H. Johnson & Co. Ltd., Stratford, London.

Briefly described, a filter-press is an apparatus for effecting filtration under pressure ; it consists in its simplest form of a number of plates having on each side a special draining surface with a raised rim all round the periphery and a central hole. A filter cloth is placed over each plate and joined round the centre hole, so that when the plates are tightly closed together hollow chambers are formed between the plates, the depth of each chamber being equal to twice the depth of the rim on the plate.

When the material to be filtered is forced in through the central hole the solids in suspension are arrested by the filter cloth and retained upon its surface in a layer of uniform thickness which gradually increases as filtration is continued owing to the accumulation of solid matter, the liquid passing through the cloth and escaping along the rills on the drainage surface

This process takes place on the cloth on both sides of the chamber and the two layers of solids gradually approach each other and finally meet in the centre of the chamber ; when this

occurs no further space is available for the introduction of more unfiltered material and the double thickness of deposit compacted into a cake of a greater or less degree of hardness, according to the terminal pressure applied, must be removed.

The rapidity with which a liquid is separated from a solid during filtration, known as the rate of filtration, depends primarily upon the force with which it is urged through the filtering medium and the resistance which is offered by the medium to the flow.

With many substances an increase of the force urging filtration results in an increase in the quantity of liquid filtered in a given time, but this rule is by no means universal, and cases frequently occur in which an increase of pressure, particularly when it is suddenly or rapidly applied, actually diminishes the rate of filtration.

It may be broadly stated that for every substance there is a particular rate of flow and hence a corresponding pressure beyond which no increased useful effect results commensurate with the increased force applied.

This may be explained by the fact that, in general, the real filtering medium is not the cloth on which the deposit is formed, but the deposit itself, and that as the pressure is increased the deposit becomes denser and more compacted, offering a greater resistance to the passage of the liquid which more than counterbalances the tendency to an increased rate of flow caused by the higher pressure.

From this it will be apparent that there is a limit, not only to the rate of deposition to secure the most favourable permeable condition of the substance deposited, but also a maximum thickness of deposit beyond which useful filtration practically ceases.

One of the most important conditions to determine in adapting a filter-press to its work is the maximum thickness of chamber which can be employed. This will be exactly twice the thickness of the deposit which limits the useful rate of filtration. Such a dimension varies widely not only with different substances but even with bodies, or precipitates, of identical composition. In the latter case the differences

consist of difference in physical condition depending largely upon the method of production.

Time and temperature, as well as the state of dilution of the medium in which the substance is suspended, all play an important part, in addition to the nature and physical condition of the menstruum in which the solid is suspended.

Filter-presses may be divided into two main types: the recessed plates and those with flush plates and distance frames.

In the recessed plate type the distinctive feature is the raised rim which stands up above the drainage surface on both sides of the plate. When the plates are in position a chamber is formed between every two plates, the depth of which is equal to twice the depth of the recess of the plate. A hole is provided in each plate through which the material to be filtered enters the chambers and a filter cloth is hung over each plate with a hole in each half to correspond with the feed hole of the plate, the joint around which is made with a clip.

When it is desired to avoid the use of cloth clips the cloth is made in two pieces sewn together round the hole; one-half of the cloth is then passed through the feed hole in the plate and the two halves are either tied together over the top of the plate or held by suitable hooks.

Recessed filter-presses usually have the feed inlet placed centrally, but some have a bottom corner inlet, and this enables the liquid contents to be drained out before opening the press.

The flush plate and distance frame type of filter-press has plates on which the faced joint surfaces are practically flush with the filtering surfaces, the chambers being formed by frames, also having faced surfaces corresponding to those on the plates and placed alternately with the plates, so that the thickness of the distance frames determines the depth of the chambers.

The filter cloths are placed and hang over the plates so that each chamber has a cloth on both sides. The inlet for the material is through a passage having ports communicating to the interior of the distance frames.

There are two main groups into which flush plate and distance frame presses may be divided, one having the passages

formed by eyes within the joint surfaces of the plates and frames, and the second having the passages in lugs external to the joint surfaces.

The latter type has a distinct advantage over the former in that no holes are required in the cloths, which are simply plain lengths cut from the roll; consequently much of the time and labour necessary to adjust the holes of the cloth to correspond with those on the plates is saved.

The filter-press is designed for the rapid filtration of yeast and is of the flush plate and distance frame type with a feed inlet passage on the top left-hand corner and filtrate discharge on the right bottom corner. The filtrate is discharged by means of a passage common to all the chambers from which the filtrate is discharged without exposure. Both these passages are external to the joint surfaces of the plates and frames so that no holes are required in the cloths.

The joints on the external passages are made with rubber rings and the press is tightened by means of a central screw which gives a more evenly distributed pressure on the joint surfaces and more uniform strains on the follower than any other method of tightening.

Yeast Cans. When the comparatively dry yeast is discharged from the filter-press it is moistened with water to the commercial consistency and then packed in bulk containers in which it is stored in the icehouse. The most sanitary and durable container is a sheet metal can, wired round the top and reinforced around the bottom circumference with heavy galvanized strap iron to keep the can at least $\frac{1}{2}$ inch off the floor.

A convenient sized can is 16 inches in diameter and 14 inches deep, furnished with a loose fitting cover that protects the yeast when the cans are stacked. Two rigid handles, riveted and sweated to the sides, assist in handling, and to assure cleanliness and prevent yeast penetration the crevice where the bottom joins the side of the can is smoothly filled with soft solder.

Hot-water Tank. This vessel is situated at the highest point of the factory and has a minimum capacity of 1,500 gallons. A wood stave vat may be used but a boiler plate tank is more durable.

Heating precipitates some of the impurities found in service water to form a heavy sediment that must be removed at intervals, depending upon the character of the water. The cover of the tank is furnished with a manhole and cap, and when constructed of boiler plate it is completely covered with 3 inches of asbestos lagging to prevent radiation.

The water from the wort cooler is heated to 150° F. by exchanging temperature with the wort and is discharged into this tank with water from the exhaust steam heaters. The temperature of the water is increased by injecting live steam through steam silencers, automatically controlled by a capsule temperature regulator set at 180° F.

The hot-water service is reticulated to those points of the factory where it is required for brewing and cleaning; its primary object, however, is to supply sufficient hot water for sparging the grains in the filter tank. An overflow discharges any excess from the tank to the boiler feed supply.

Refrigeration is the process of abstracting heat from any substance; and if water or any other suitable medium is available at a lower temperature than is desired in the substance to be cooled, such water or medium may be used to effect the cooling directly and without a machine.

Apparatus for producing the same effect mechanically may be divided into two classes, one of which depends upon the utilization of the latent heat of liquefaction and vaporization and the other upon the mutual convertibility of heat and work.

The icehouse in which the yeast is stored is chilled by ammonia expansion coils of ample capacity over which the air of the chamber is continually circulated by means of a ventilating fan, and frequent starting of the compressor is avoided by providing an abundant storage of chilled brine in a brine tank 10 feet long, 4 feet deep and 12 inches wide, set in the middle of the room, through which the return tube from the expansion coil passes before it leaves the room.

The capacity of the ammonia compressor is expressed in tons of refrigeration computed from the amount of heat absorbed in melting 1 ton of ice at 32° F. into 1 ton of water at 32° F. One pound of ice at 32° F. absorbs 143·5 British thermal units in melting to water at 32° F., and 1 ton of

refrigeration = 143·5 × 2,000 lb. = 287,000 B.Th.U. The total amount of refrigeration required, or the capacity of the machine per twenty-four hours, will be influenced by local conditions, by the disposition of the plant and by insulation efficiency; but the known amount required for the example brew may be estimated as follows :—

The cultivation of 1 lb. of yeast develops 729 B.Th.U. and one ton = 729 × 2,240	1,632,960
870 gallons of yeast cream chilled from 86° F. to 45° F. = 8,700 × 1·2 × 41° F. . . .	428,040
1,810 gallons of store wort chilled from 80° F. to 45° F. = 18,100 × 35° F.	633,500
2,600 lb. of yeast reduced from 65° F. to 40° F. = 2,600 × 25° F.	65,000
Total B.Th.U.	2,759,500

Pumps. Most of the material transported in the yeast factory is in liquid form and the pumps and pipe lines used to conduct these liquids from one part of the factory to another form a very important section of the plant.

When a number of pumps are used in one establishment maintenance charges are reduced if they are standardized; and in this industry centrifugal pumps are recommended for their flexibility and economy and the ease with which any particular design may be standardized in the factory.

Factory Cleanliness. This in every unit, section and department of the factory is an essential condition for the production of compressed yeast.

Cleanliness is a relative term, very loosely applied, and the industrial standard of cleanliness is, all too often, the lowest under which an industry can exist. The cheap grades of vinegar, for instance, are still produced under appalling conditions in surroundings that often can only be described as revolting, simply because acetic acid bacteria has the power to suppress infection and the energy to continue oxidation under adverse conditions.

Yeast, on the other hand, is susceptible and easily suppressed by wild yeasts and moulds; and its cultivation demands the highest degree of bacteriological cleanliness and sterility in everything with which it is brought in contact. This also demands cleanliness of a very high order on the walls, floors and ceilings of the factory building.

The ease with which a surface can be cleaned depends upon the porosity of the material and the smoothness of its surface. On hard and impervious surfaces impurities merely adhere and may be easily removed, but on porous materials these impurities will penetrate the pores and are only partially removed by a tedious process of scouring and cleaning.

For this reason structural steel is used for tank and vat supports. Floors are reduced to the minimum area, and where wood flooring must be used it is covered with an impervious layer of bitumen or similar material. Walls should be tiled or rendered with cement trowelled to a smooth finish and covered with a hard enamel paint that dries with a glossy surface. Sheet metal walls simplify cleaning operations.

Since micro-organisms require moisture for their sustenance, a cheap and powerful antiseptic is the absence of moisture. The resistance of dry substances to decomposition is shown by the length of time dry spent grains will keep in comparison with the rapid putrefaction of wet spent grains.

Condensation from the moisture-laden atmosphere of the yeast factory provides ideal breeding grounds for numerous types of infective micro-organisms, whilst dust from the milling and malt culms provides the food. Continual vigilance is therefore required to keep the factory reasonably clean, and the intelligent application of suitable antiseptics is essential in order to destroy all forms of life in vessels, pipe lines and fittings.

Heat is one of the best antiseptics, and also the most economical. It is applied in both the dry and wet forms, but in the yeast factory moist heat is used in the form of saturated steam, and no safer general antiseptic is available owing to its effect on cell proteins. There is an analogy between the antiseptic value of heat and the coagulation of proteins which is reflected in the difficulty with which spores are destroyed when

compared with vegetative cells which contain much more water and succumb more readily to the action of dry heat.

Spores contain hygroscopic moisture that evaporates and leaves a dry cell that resists the effect of dry heat for some considerable time. The condition of the steam used in sterilization is therefore important because steam at 212° F. with only 80% saturation takes five times as long to kill bacteria as does saturated steam, and with 70% saturation it requires twenty times as long.

The correlation between the temperature of steam and its saturation is an important factor. For instance, when steam is superheated it is altered in two ways. The temperature is increased, which increases its antiseptic power, but its saturation is reduced, which destroys its value as a disinfectant. In this case the destruction of all forms of life in vessels and utensils is achieved by the combined effect of steam and formaldehyde, the action of which is generally considered to be an action upon the protoplasm of the cells. The formaldehyde is supposed to combine chemically with the cell plasma to produce compounds which interfere with the vital processes and cause death; this action is accelerated until it becomes almost instantaneous in the presence of steam.

Formaldehyde is sold as a liquid in a 40% solution under the name of formalin; it also polymerizes to a white crystalline substance and is sold in the dry state. In this form it is quite inoffensive and easily handled, and is changed to the gaseous form by gentle heating. It does not attack metal or woodwork, and is preferable to many other chemical antiseptics. It kills spores in a short time in a 1 in 1,000 dilution; or the saturated solution may be evaporated directly: but its effectiveness is greatest when it is diffused in the hot moisture-saturated atmosphere produced by passing steam through the 40% solution.

The Laboratory

Special Apparatus Required. The yeast factory laboratory should include equipment and apparatus necessary for general analysis and process control, supplemented by mycological apparatus for the isolation and growth of yeast pure cultures.

Flasks of different designs are required for the preservation of giant colonies on solid media ; and for propagating yeast pure cultures in sweet wort.

Platinum wire is used in pure culture work in the form of needles and loops, made by fusing a 3-inch length of 26-gauge wire into the end of a suitable piece of glass rod ; the wire is then either bent to shape or left straight as required. In plating cultures standard loops which deliver the same amount of liquid are used. A loop 3 mm. in diameter will carry a drop of media amounting to about 0·02 c.c. according to the angle at which it is taken from the liquid.

The incubator is a double-walled zinc or copper chest covered with some impermeable material and fitted with double doors to prevent radiation. The space between the double walls is filled with water or with a mixture of water and glycerine. Gas heating has been superseded by electrical heating with thermostat control.

The sterile chamber is a glass case approximately 30 inches long, 24 inches wide and 24 inches deep. A plate glass supports the four wood corner uprights, into which the glass sides and top are fitted. The glass front of the chamber is a counter-poised sliding door opening vertically, and this opening is protected from convection air currents by a cover of sheet rubber in which two elastic bordered openings are provided for the hands of the operator. The chamber is sterilized by spraying the interior with a 1 in 1,000 solution of mercuric chloride, and closing it for an interval of thirty minutes before use.

The hot-air oven is used for the dry sterilization of small flasks, cotton-wool and petri dishes. It is made of sheet metal with double or treble walls, and its construction is such that after the temperature has been raised to 350° F. this temperature is maintained for a long time after the source of heat is removed.

The Microscope. The compressed yeast industry was not only founded upon the discoveries of the microscope, but its continuance depends entirely upon the skilled use of this delicate piece of apparatus. The chemist in the yeast industry will possess a knowledge of the microscope and its application

and the details given here will, therefore, be restricted to special requirements.

An essential fitting, for the examination of yeast cultures, is a mechanical stage that enables the whole of a specimen to be examined over its entire surface. This attachment should be provided with scales and verniers so that any position in a specimen can be recorded and the same position found at any future time by setting the stage to the same reading.

The object glasses and eyepieces are of such importance in the performance of a microscope that their use and selection receives very careful consideration. Their glass surfaces cannot be touched by the fingers without leaving a smear of grease and every care is necessary in cleaning the lenses as they are easily marred or scratched. Dirt can be removed from the eyepiece with a soft piece of silk moistened with a little xylol or alcohol. The lens which requires most cleaning is the front of the oil immersion which is necessarily covered with cedar wood oil, but a piece of filter paper moistened with xylol and wiped lightly over the front surface will remove the oil without rubbing.

Modern object glasses are made in different powers to different magnifications in the primary image. The name $\frac{2}{3}$, $\frac{1}{6}$ or $\frac{1}{12}$ inch, as applied to an object glass, represents its focal length and indicates its magnifying power. If an ordinary single lens of 2-inch focal length is used as a hand magnifying glass it has to be placed about 2 inches from an object to give a clear image, and the $\frac{2}{3}$, $\frac{1}{6}$ and $\frac{1}{12}$ inch objectives require to be placed about these respective distances from the object when in use, thus the higher the magnifying power of a lens the closer it must be to the object.

The three above objectives are most suitable for the yeast laboratory. The $\frac{2}{3}$-inch object glass gives a maximum field of view of 0·08 inch (2 mm.). It is a useful low-power lens for searching specimens to be examined later with a high power.

The $\frac{1}{6}$-inch (4 mm.) object glass has a maximum field of view of 0·02 inch (0·5 mm.) and has a working distance of 0·024 inch. It is the universal high-power lens, and is used almost exclusively for routine yeast examination.

The $\frac{1}{12}$-inch oil immersion object glass has a maximum field

of view of 0·0085 inch (0·2 mm.) and a working distance of 0·01 inch. This is the high-power lens used when it is necessary to see the finest details that can be observed with any microscope. It is the object glass that reaches the highest limit yet obtained in microscopic vision.

Holding an object under the microscope for examination calls for various appliances according to the nature of the object. The universal method consists of placing the object between a glass slip and a thin cover-glass. These slips are made 3 inches long and 1 inch wide, the thickness varying between 0·5 and 1·5 mm.

Cover glass is a specially thin form of glass prepared for use with the microscope. It is made in squares or circles of $\frac{5}{8}$–$\frac{7}{8}$ inch in diameter and in three thicknesses, 0·15 mm., 0·2 mm. and 0·25 mm. The thickness varies about 20% in different individual pieces, and absolute uniformity of thickness can only be obtained by selection. An important factor in the best performance of an object glass is the thickness of the cover-glass between the object and the lens.

The illumination of the object is almost as important as the quality of the lenses; and the proper use of the sub-stage condenser is essential for correct observation. A bad lens can never give a perfect image, but a good lens will only give a good image when the illumination is perfect. When yeast is examined in wort or water, a drop is placed on the glass slip and a cover-glass placed over it at an angle in such a manner that the cover-glass touches one side of the drop first and is then allowed to fall in a way that prevents air bubbles being enclosed.

Hydrometers. This instrument is in general use for determining the specific gravities of liquids. Different kinds of hydrometers bear names according to the kinds of liquids for which they are used. Saccharometers, for instance, are a special kind used to indicate the amount of sugar in saccharine solutions or the amount of brewers' extract in worts.

In addition to the Balling saccharometer the so-called corrected Balling, or Brix scale, saccharometer is often used in distillery practice. When cane sugar is dissolved in water a contraction takes place, and this contraction varies with

the concentration. Brix calculated these contractions and introduced his instrument with the corresponding corrections.

The graduation of these instruments can be verified by dissolving pure cane sugar in distilled water in the percentages indicated. The most accurate saccharometers have the scale graduated in tenths of a degree, and the range of degrees indicated by each instrument should be as limited as possible. The ranges most suited for yeast factory practice are from 0° to 5° Balling, for differential fermentations, 0° to 14° Balling, for store worts and spirit fermentations, and from 10° to 20° Balling for mash tun worts.

Thermometers. In every section of the yeast factory accurate thermometers are essential for process control.

Hydrogen-Ion Concentration

The titration acidity of a solution is determined by finding the amount of soda solution of a given strength that must be added to neutralize the acid. Normal solutions of hydrochloric, acetic and lactic acids all take the same amount of normal soda solution to neutralize them; but if they were tasted the hydrochloric acid solution would taste much more sour than the other equally strong solutions.

This is explained by the fact that different acids differ in their tendency to throw off hydrogen ions and it is only these ions that taste sour. An acid such as hydrochloric acid will at once throw off all its hydrogen ions when dissolved in water; lactic acid, on the other hand, and more particularly acetic acid, keep the greater part of theirs in reserve: the hydrochloric acid therefore is very sour to the taste, the other acids only slightly so, although all three are present in the same degree of strength.

In many cases the estimation of acidity, or alkalinity, by ordinary methods of titration leads to results which prove to be misleading when they are applied in mycological processes, but the estimation of hydrogen-ion concentrations may be described as the estimation of the intensity of acidity because it does not consider potential hydrogen ions which are still incorporated in undissociated molecules, while titrimetric

methods liberate and estimate the potential ions, thus recording total acidity.

All living cells depend upon the maintenance of a strictly limited hydrogen-ion concentration in their surrounding medium. Yeast flourishes best between *p*H 3 and *p*H 4·5; the optimum for *B. coli* is at *p*H 5, and *B. subtilis* develops best between *p*H 4·5 and *p*H 9. The elimination of the bacterial disease known as rope in bread has been accomplished by *p*H measurements. There is also an optimum *p*H for enzyme activity, diastase for instance is most active at *p*H 5, and this concentration is necessary in the mash tun to secure the maximum conversion of starch to sugar.

Colorimetric Determination of *p*H in Fermenting Worts. This depends upon the fact that if an indicator, whose range of colour change extends over the range of hydrogen-ion concentrations to be determined, is added to a solution of unknown *p*H, a definite shade of colour depending upon the *p*H will be developed.

If the same amount of indicator is added to a series of buffer solutions of known hydrogen-ion concentration, a gradation of shades of colour is produced, and by matching the shade of the unknown sample with these its *p*H can be determined. The symbol *p*H represents the negative exponent of the hydrogen-ion concentration; it was introduced by Professor Sorensen and is known as Sorensen's constant.

The point of absolute neutrality at which the numbers of hydrogen and hydroxyl ions are equal is represented by the value *p*H 7·0. If *p*H is less than *p*H 7·0 hydrogen ions predominate. If it is greater than *p*H 7·0 hydroxyl ions are in the majority. The value *p*H 7·0 represents a hydrogen-ion concentration of one ten-millionth normal.

A deci-normal solution of a strong acid such as hydrochloric acid will be close to *p*H 1·0, or almost deci-normal to hydrogen ions, while a deci-normal solution of a strong alkali of *p*H 13·0 will contain hydrogen ions to the extent of one ten-billionth normal.

The phenomenon of buffer action materially influences *p*H determinations. For instance 1 c.c. of N/100 hydrochloric acid when added to a litre of pure water of *p*H 7·0 gives a solution of *p*H 5·0. The same amount of acid added to a

litre of wort, or any solution containing salts of weak polybasic acids, such as phosphates or carbonates, would not produce any appreciable change in *p*H.

The following abstract from Professor S. P. L. Sorensen's original treatise on "The determination and importance of hydrogen-ion concentration in enzyme processes"* describes the preparation of the standard solutions and the method used for the colorimetric determination of *p*H. The solutions required are N/10 hydrochloric acid, N/10 sodium hydroxide, N/10 molecular solution of secondary sodium citrate and a 0·7 per cent. aqueous methyl orange solution.

The tenth normal solution of hydrochloric acid is prepared by diluting pure HCl with recently distilled CO_2 free water and standardizing with sodium oxalate or borax. One gram of borax = 52·39 c.c. N/10 HCl.

The tenth normal solution of sodium hydroxide is prepared by diluting a carbonate-free sodium hydroxide solution, standardized by titrating with the N/10 HCl solution. The carbonate-free solution is prepared by dissolving 250 gm. of pure sodium hydroxide in 300 c.c. of distilled water in a glass-stoppered cylinder; the carbonate is insoluble in this concentrated solution and settles to leave a clear supernatant solution of sodium hydroxide. The absence of carbonate is confirmed by withdrawing a sample, diluting with distilled water, neutralizing to phenolphthalein with HCl, to a faint red tint and adding an excess of neutral barium chloride solution. In the absence of carbonates the colour remains undischarged.

The secondary sodium citrate solution is prepared by dissolving 21·003 gm. of pure crystallized citric acid in 200 c.c. of N/1 sodium hydroxide solution and diluting to 1 litre with distilled water.

The indicator solution is prepared by dissolving methyl orange in distilled water, the solution is allowed to stand for twenty-four hours and then filtered.

Precautions must be taken to prevent the absorption of CO_2 by any of the solutions. Recently distilled water only is used in their preparation, exposure to atmospheric contamination is avoided as much as possible and CO_2 is excluded

* "Reports from the Carlsberg Laboratory," Vol. 8, 1909–10.

from all flasks used in their preparation by drawing a current of air from a soda-lime tube through them.

After the solutions are standardized they are placed in aspirator bottles fitted with side tubulures and a doubly perforated stopper at the top (see Fig. 27). In one top perforation a soda-lime tube is placed, and in the other a glass tube bent twice at right angles connects with the top of a 50 c.c. burette fitted with side tube, jet, and pinch cock. From the single perforated stopper in the side tubulure of the aspirator bottle a glass tube bent at right angles is connected to the filling tube of the burette, with a pinch cock on the rubber connecting tube to regulate the supply of reagent to the burette. The aspirator bottles are most conveniently arranged when they are placed side by side upon a shelf about 3 feet above the desk.

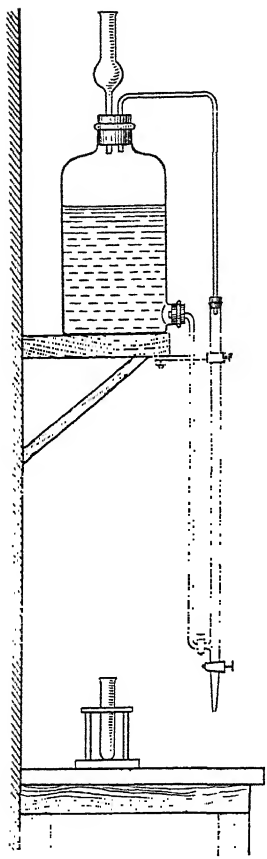

FIG. 27. Apparatus, with burette, arranged for storing the buffer solution.

The stock of methyl orange is stored in a glass-stoppered bottle from which a dropping bottle of 200 c.c. capacity is filled as required.

A number of boiling-tubes are required and they should be about 1 inch in diameter and 6 inches long, but as uniform as possible with a maximum variation in diameter not exceeding 0·25 mm. The tubes are closed with the best quality tight-fitting corks, and before the tubes are filled with buffer solution they are steam sterilized for sixty minutes.

The indicator tubes are prepared for use by measuring the quantities of buffer solutions shown in the following table

direct into the tubes from the respective burettes and then adding 20 drops of the methyl orange solution to each tube.

*p*H	Cubic centimetres N/10 HCl solution	Cubic centimetres Citrate solution.	*p*H	Cubic centimetres N/10 HCl solution	Cubic centimetres Citrate solution
2·0	13·7	6·3	4·0	8·8	11·2
2·2	13·4	6·6	4·2	7·8	12·2
2·4	13·1	6·9	4·4	6·4	13·6
2·6	12·7	7·3	4·6	4·6	15·4
2·8	12·3	7·7	4·8	2·4	17·6
3·0	11·9	8·1		c.c. N/10	
3·2	11·4	8·6		NaOH	
3·4	10·9	9·1	5·0	0·8	19·2
3·6	10·3	9·7	5·2	3·0	17·0
3·8	9·6	10·4	5·4	4·8	15·2

Each tube contains 20 c.c. of the buffer solution plus 1 c.c. of the indicator, and is plainly marked with its respective value. The tubes are immediately closed with the sterile corks, and if the work has been carried out expeditiously they may be used for a considerable period before refilling becomes necessary.

The tubes are placed in a wood stand to determine the *p*H of solutions. This may be cut from a solid block of wood and drilled with the holes slightly larger than the tubes they support ; sight holes are then drilled at right angles to the test-tube holes similar to the positions shown in Fig. 28, which indicates the shape and dimensions of this stand, known as the comparator block.

To determine the *p*H of fermenting wort, 20 c.c. is filtered into a calibrated test-tube, exactly the same size as the buffer tubes, 20 drops of methyl orange solution is added and, after shaking, the tube is placed in the orifice marked A on the diagram, a test-tube containing distilled water is placed in the orifice marked B, a test-tube containing fermenting wort filtered bright is placed in D.

The standard buffer solutions vary in colour from bright red (*p*H 2·0) to yellow (*p*H 5·4) and a tube is selected that

matches the colour of the test solution in orifice A and is placed in C. The tubes are now viewed through the sight holes, and the tube of standard solution is changed if necessary until the colours are exactly matched. The *p*H of the wort under examination corresponds to that shown on the standard tube of buffer solution.

In this as in all colorimetric determinations every effort is made to obtain uniform conditions of illumination ; excellent results are obtained by using an opal glass electric bulb, the

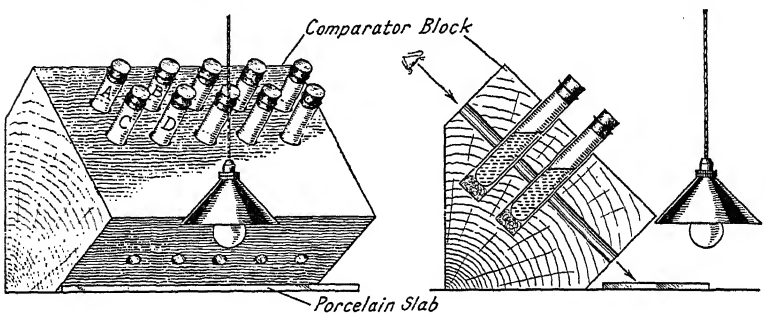

FIG. 28. The comparator.

Sectional view of the comparator showing tubes and the line of vision.

Tube A contains from 20 to 30 c.c. of the filtered wort without the addition of indicator solution. Tube C contains the standard buffer solution. This tube is changed until one is found to match the colour of the wort in tube B. Tube B contains 20 c.c. of the filtered wort and 20 drops of the indicator solution. Tube D contains distilled water.

light from which is reflected from a porcelain slab placed at the back of the comparator.

There are numerous types of apparatus sold under trade names for the colorimetric determination of *p*H in which tinted glass or permanent buffer solutions are employed, but the indicating and recording potentiometer is a refinement that simplifies the *p*H control of all mycological processes, and its application to the fermentation industry effects a considerable saving in time and labour.

The Formalin Titration. For the estimation of assimilable nitrogen in fermenting worts.

Sorensen introduced the formalin titration method for determining the amount of nitrogen in a solution, which enters

into reaction with formaldehyde. Sufficient alkali is first added to the solution to render it neutral to phenolphthalein ; neutral formalin is then added and, owing to the reaction, the solution becomes strongly acid. The additional amount of alkali then necessary to neutralize this acidity is the measure of the nitrogen.

In applying this method to determine the amount of assimilable nitrogen in fermenting wort a quantity of a 40% solution of formaldehyde is neutralized by the addition of normal NaOH until its colour is a faint pink to phenolphthalein.

The nitrogen is then determined and expressed as the formalin number by neutralizing 100 c.c. of the wort under examination with N/1 NaOH until the colour is a rose pink to phenolphthalein. Ten cubic centimetres of the neutral formalin is added, and it immediately decolorizes the solution by the acid formed in the reaction that follows. The solution is then titrated with N/10 NaOH to the same rose pink colour. The formalin number is defined as the number of cubic centimetres of N/10 NaOH required to restore the colour of the solution.

This examination is conveniently combined with the acid titration in fermentation control, the same sample of wort being used for both determinations ; 100 c.c. of the wort is measured into a graduated cylinder, transferred to a beaker and titrated with N/1 NaOH and neutral litmus paper. The number of cubic centimetres of alkali used is noted as degrees of acidity. The neutral solution is then further titrated to the required tint with phenolphthalein and used for the formalin titration.

The Preparation of Standard Solutions. Soxhlets modification of Fehling's solution is prepared by mixing equal volumes of the following solutions :

Copper sulphate solution. Dissolve 34·639 gm. of copper sulphate, free from iron and moisture, in distilled water, dilute to 500 c.c. and filter through prepared asbestos.

Alkaline tartrate solution. Dissolve 173 gm. of rochelle salts (potassium sodium tartrate) and 50 gm. of sodium hydroxide in distilled water, dilute to 500 c.c., allow to stand for two days and filter through prepared asbestos.

The above individual solutions keep indefinitely, but the prepared solution suffers decomposition so that a reduction occurs on boiling. For this reason the solutions are mixed just before being used. The addition of the rochelle salt is for the purpose of dissolving the cupric hydroxide that would otherwise be precipitated by mixing the solutions. It is advisable to test the condition of Fehling's solution before use by diluting a quantity with water and heating it to boiling point for a few minutes; it should remain perfectly clear after this treatment.

Fehling's solution is not accepted as a standard solution until it is tested against sugar. Its true oxidizing power with invert sugar is determined by dissolving 4·75 gm. of pure cane sugar in 75 c.c. of water, add 10 c.c. of HCl, specific gravity 1·188, and set aside to invert for twenty-four hours at room temperature. Neutralize the acid with sodium hydroxide and dilute to 1 litre. Ten cubic centimetres of this solution contains 0·05 gm. of invert sugar which should reduce 10 c.c. of the reagent.

In standardizing the reagent the approximate volume of the sugar solution required to reduce the Fehling's solution is determined by a preliminary titration. Ten cubic centimetres of Fehling's solution is measured into a 200-c.c. boiling flask and heated to boiling over a Bunsen flame; 5 c.c. of the sugar solution is run in from a burette, boiled and then succeeding small portions are added, boiling after each addition. When the colour change indicates the approach of the end point the flask is heated over a wire gauze and one drop of a 1% solution of methylene blue is added to the flask. Small portions of the sugar solution are then added and boiled until the blue colour is discharged.

The titration is then repeated using nearly the full volume of the sugar solution required so that only a few tenths of a cubic centimetre is necessary to complete the titration. This indicator is so sensitive that the end point can be determined to within less than one drop of sugar solution.

The reducing sugar in wort is volumetrically estimated by diluting the wort with water until it contains about 0·5% sugar and placing it in a burette. Exactly 10 c.c. of the

Fehling's solution is measured into a boiling flask, heated to boiling point and the boiling continued until the titration is completed. The sugar solution is added rapidly as possible, and when the first colour change is noticed one drop of methylene blue is added and the cautious addition of sugar solution resumed until the blue colour is discharged.

The amount of sugar necessary to reduce a given volume of Fehling's solution varies with the kind of sugar; for instance, 0·05 gm. of dextrose, or invert sugar, or 0·0678 gm. of milk sugar, or 0·0807 gm. of maltose will reduce 10 c.c. of Fehling's solution prepared according to the above directions.

Standard solutions may be made of any strength suitable for the particular estimations to which they may be applied.

Normal solutions. The term normal is used to indicate a solution of which 1 litre contains the gram equivalent of the dissolved substance; by the term equivalent is understood the weight in grams of the substance which is chemically equivalent to 1 gm. of hydrogen.

Hydrochloric acid, for instance, is a monobasic acid and a normal solution contains 36·7 gm. per litre, whereas a normal solution of sulphuric acid, a dibasic acid, contains 98·8 + 2 = 49·4 gm. per litre.

Similarly a normal solution of caustic soda contains 40·01 gm. per litre, whereas normal sodium carbonate contains 106 + 2 = 53 gm. per litre. Normal solutions are distinguished by the letter N and solutions of fractional strength are denoted by N/2, N/10, N/100.

Many substances are recommended for use as standards in alkalimetry and acidemetry, and owing to the purity of samples that can be readily obtained, sodium carbonate is one in common use and is prepared by heating the bicarbonate at 360° F. until constant in weight.

Oxalic acid is frequently used, but borax is readily obtained in the pure state, and the rapidity and ease of manipulation recommends this substance as a standardizing agent. Four grams of chemically pure dry borax are weighed, dissolved in 60 c.c. of hot distilled water, cooled, one drop of methyl orange added and the solution titrated with normal hydro-

chloric acid. One gram of borax equals 5·239 c.c. of N/1 HCl.

Methods of Analysis

Extract in Raw Grain. *Estimation by direct acid conversion.* The results obtained by this method are generally much higher than that obtained in practice from the same material in the factory owing to the hemicellulose and carbohydrates, that are not acted upon by diastase, but are capable of acid conversion to sugar and thus are reckoned in with the starches.

The substance is finely divided, and 5 gm. are exhausted on a filter with ether by washing with five successive portions of 10 c.c. each ; the residue is then washed with 50 c.c. of 10% alcohol and again with 10 c.c. of 95% alcohol. It is then transferred to a flask by washing with 200 c.c. of an aqueous solution containing 20 c.c. of HCl (specific gravity 1·125). The flask is connected to a reflux condenser and heated on a boiling water bath for two and a half hours. It is then cooled, neutralized with NaOH, clarified with alumina cream, the volume made up to 500 c.c. and filtered. The dextrose is determined in an aliquot portion gravimetrically, or by titration with 10 c.c. portions of Fehling's solution ; the percentage of sugar found may be calculated to starch by the factor 0·9.

Estimation by Diastatic Conversion. This method gives results which, although generally high, are nearer the extract obtained in brewing ; the hemicellulose is not converted, the starch alone being acted upon by the diastase and converted into maltose and dextrin ; these substances in turn are converted by acid hydrolysis into dextrose.

The substance is finely crushed and 5 gm. are exhausted with ether and alcohol, as in the direct acid conversion method ; the residue is washed into a beaker with 500 c.c. of water and immersed in a boiling-water bath for fifteen minutes or until completely gelatinized, stirring all the while. Cool to 55° C., add 20 c.c. of malt extract and digest at 55° C. for one hour. Heat again to boiling and boil for fifteen minutes ; replace the water lost by evaporation, cool to 55° C., digest as before with

20 c.c. of malt extract for one hour or until the residue shows no starch when treated with iodine.

Cool, make up to 250 c.c., filter, pipette 200 c.c. into the flask, add 20 c.c. HCl (specific gravity 1·125), and proceed as in the direct acid conversion method. Correct for the copper-reducing power of the malt extract as follows :

Preparation of the malt extract. Digest 10 gm. of freshly ground malt with 200 c.c. of water for two hours at room temperature, shake occasionally, and filter. Determine the amount of dextrose in a given volume of the extract after heating with acid and treating as in the above analysis and make the requisite correction.

Extract in Raw Grain. *Brown's method.* The procedure introduced by H. Brown is frequently used in determining the extract in raw grain. Of the finely ground cereal 5 gm. are extracted in a suitable apparatus with alcohol (specific gravity 0·9) for three hours, transferred to a beaker containing 100 c.c. of water, and boiled.

After cooling to 57° C., 10 c.c. of malt extract (diastatic activity 60° Lintner) are added and the conversion allowed to proceed for sixty minutes ; the solution is boiled, filtered into a 200 c.c. flask, the residue washed and the volume of the filtrate adjusted to the mark after cooling.

The cupric reduction of 20 c.c. is determined gravimetrically and the percentage of maltose calculated from the copper reduced, after making the correction for the reduction due to the malt extract ; 84·4 parts of maltose corresponds to 100 parts of starch.

Moisture in Cereals. About 5 gm. of the crushed grain is weighed in a shallow dish and placed in a boiling-water oven for five hours, cooled in a desiccator and re-weighed. The difference in weight is taken as the moisture content and calculated as a percentage of the grain.

Extract in Malted Grain. Kusserow's method. Crush about 30 gm. of the malt and weigh exactly 20 gm., place in a 250 c.c. tared flask, add 100 c.c. of water at 133° F., and after shaking stand the flask in a water bath at 158° F. for exactly one hour, shaking at intervals of ten minutes. Remove the flask, make the weight of the mash up to 200 gm. by the

addition of cold water, stir and filter into a graduated cylinder, return the first cloudy filtrate until it runs clear. The wort is cooled to 65° F., its specific gravity determined and from this the degree Balling ascertained by reference to the tables. The extract is calculated by the following formula in which A = gravity degrees Balling, Aq = moisture content of the malt.

$$\text{Extract in malt} = \frac{A}{100 - A}(900 - Aq).$$

Moisture in Malt. About 5 gm. of the sample are weighed in a weighing bottle and heated in a boiling-water oven for three hours, cooled in a desiccator and re-weighed. The loss in weight is taken as the moisture, and this may be as low as 0·5% from malt fresh from the kiln or as high as 2·5% from the storage bins. If the moisture exceeds 3·0% the malt is said to be slack.

Diastatic Activity of Malted Grains. *Lintner value.* Exactly 25 gm. of malt is crushed and extracted with 500 c.c. of distilled water for three hours at 70° F., stirring every half hour; it is then filtered through a ribbed filter paper and the first 100 c.c. of the filtrate rejected; 3 c.c. of the bright filtrate is allowed to act on 100 c.c. of a 2% solution of soluble starch at 70° F. for one hour in a 200 c.c. flask; 10 c.c. of N/10 NaOH is then added to stop further diastatic action, the liquid is cooled to 60° F., diluted to 200 c.c. with distilled water well shaken and titrated with 5 c.c. portions of Fehling's solution, using methylene blue as an internal indicator.

The result is calculated by the following formula in which A = diastatic activity, X = the number of cubic centimetres of the extract contained in each 100 c.c. of the fully diluted starch conversion liquid, and Y = the number of cubic centimetres of the same liquid required for the reduction of 5 c.c. of Fehling's solution.

$$A = \frac{1{,}000}{XY}$$

In this determination the result is accurate only when the maltose produced does not exceed 40% of the starch used. In testing malts with a diastatic activity not exceeding 50°

Lintner, 3 c.c. of the malt extract is used as indicated, but in the examination of malts of greater activity the amount of malt extract must be reduced to 2 c.c. or even less.

The soluble starch is prepared by digesting 500 gm. of purified potato starch with 1,000 c.c. of a dilute hydrochloric acid solution (specific gravity 1·037) at room temperature for seven days. The starch is then washed with copious volumes of water by decantation, and finally with distilled water until free from chlorides ; it is then filtered, dried on a porous plate, ground in a mortar and rubbed through a fine hair sieve.

Soluble starch differs from the original starch in that it dissolves to a clear solution in warm or hot water and is not completely precipitated by half saturation with ammonium sulphate in five minutes. It should be perfectly mobile with a negligible reducing action on Fehling's solution and neutral to litmus. The distilled water used in preparing the starch must be pure and free from the traces of ammonia and nitrites frequently present in commercial distilled water.

The Estimation of Alcohol. Before the amount of alcohol present in an aqueous solution can be determined it must be separated by distillation from any foreign matter that may also be present in the solution.

The alcohol content in fermented cereal extracts is found by measuring 100 c.c. at 60° F. in a graduated volumetric flask, it is transferred to a 1,000 c.c. distillation flask, diluted to about 200 c.c., neutralized with a slight excess of NaOH to litmus, and frothing prevented by the addition of paraffin wax. The alcohol is distilled and boiling continued until about 80 c.c. has passed over ; this is cooled to 60° F. and made up to the mark of the original volume of wort taken with distilled water.

The specific gravity of the distillate is found by weighing 50 c.c. in a density bottle and comparing the weight found with an equal volume of water at the same temperature. The density bottle is a small flat-bottomed flask fitted with a ground-glass stopper having a flat top and pierced with a hole of capillary bore that allows the flask to be filled and stoppered without enclosing air bubbles. The density bottle is carefully cleaned, filled with distilled water at 60° F. and allowed to

stand in a water bath at the same temperature for fifteen minutes. The stopper is carefully inserted and the flask dried and weighed. After emptying, it is rinsed with alcohol, then with ether, the ether fumes are removed and the perfectly dry flask is weighed.

The weight of the water is found by subtracting the weight of the empty flask from its weight when full.

The weight of the alcoholic distillate is found by filling and weighing the flask with the sample at 60° F. and subtracting the weight of the flask.

The specific gravity of the distillate is calculated from the respective weights of the water and distillate and the percentage of alcohol is found by reference to the alcoholometric tables.

Yeast Tests. The routine examination of compressed yeast. A sample of yeast from each brew is taken from the filter press and subjected to routine tests in the laboratory and, if convenient, to a practical bakehouse trial. These tests are of value in estimating the uniformity, dough-raising power, and fermentative energy of yeast grown in each brew, and enable interesting comparisons to be made when the results of the tests are recorded in tabulated form.

Colour. Compressed yeast is uniformly cream in colour without streaks or bands. A slight flesh tint is often observed in yeast fresh from the press, but in a few hours this changes to the normal pale cream tint. A grey colour invariably indicates weakness and a low nitrogen content.

Odour. The odour of yeast is generally ethereal and pleasant. Stale yeast develops a sharp sickly smell reminiscent of decomposing potatoes.

Fracture. Commercial yeast breaks with a clean fracture. Stickiness indicates either excessive moisture, infection, or alkalinity. When exposed to room temperature for a few days yeast loses some of its adherent moisture and crumbles easily. If the yeast is cool this is not detrimental, and it may be restored to its former consistency by the addition of cold water.

Microscopic Examination. The percentage of dead or weakened cells is determined by microscopic examination in

which the stained and unstained cells are counted by means of the hæmacytometer. Living protoplasm does not stain, whereas dead plasma readily absorbs many dyes, and by this means dead or weakened cells are detected by the intensity of the colours they assume. A little of the yeast is rubbed up with distilled water and, after adding one drop of methylene blue or carbol fuchsin, the cells are examined under a magnification of 600 diameters.

The cells should be uniform in shape and size, free from bacterial infection or admixture of foreign matter of any description. The number of stained cells should not exceed 0·5% ; the presence of unstained cells with granulated plasma, or abnormal shapes, indicates high temperatures during fermentation.

Pelleted Yeast. When pure yeast is mixed with water it forms a smooth cream of suspended cells without sediment, but pelleted yeast does not form a suspension, and leaves a heavy sediment of yeast-like granules. This condition is due to infection of either the wort or seed yeast with a mould of the monilia candida species, which thrives in turbulently aerated worts and, if not eradicated, may entirely suppress yeast growth.

In the examination of yeast for pelleted infection 50 gm. are mixed with 500 c.c. of water and allowed to stand for five minutes ; the suspension is decanted, the sediment stained and examined under a magnification of 500 diameters. When pellets are present the field will show a number of normal healthy cells separated from an agglomeration of closely packed yeast-like cells, resembling a cluster of grapes, surrounding the stained, branched, mycelium of the parent infecting cell.

Dough Test. This test is universally applied to determine the dough-raising power of yeast ; but it has a local application only. If the result of any yeast test is to be compared with similar tests conducted elsewhere it is essential that all other factors should be constant with the yeast as the only variant ; at present this condition is impossible owing to the difficulty of standardising the flour used in the tests.

The modifications that occur in the ageing of flour naturally

influences the dough test, and the only way in which this influence may be minimized to any extent is by maintaining a stock of test flour of such an amount that not more than 5% is used per week. This flour is stored in a tumbling barrel and its weight is kept constant by replacing the flour taken for each test with new flour and securing a perfect blend by rotating the barrel for a few minutes.

The dough test measures the time taken by the yeast to expand a small piece of dough to a mass of definite volume; measured in a dough mould made of sheet copper to the following dimensions:

Top length 155 mm. Bottom length 136 mm. Top width 100 mm. Bottom width 87 mm. Inside vertical depth 87 mm.

A sheet metal bar is cut in such a manner that when placed across the top middle of the mould its bottom edge dips about 13 mm. inside. The mould is then placed on a level surface and calibrated to a capacity, at the bottom of the bar, of exactly 1,000 c.c. with water at 86° F.

The test dough is made of the following materials: Cane sugar 2 gm.; salt, 8 gm.; flour, 280 gm.; distilled water, 160 c.c.; yeast, 10 gm.

The flour, contained in a 10-inch evaporating dish, is placed in an incubator at 86° F. for thirty minutes before use, and the salt and sugar is dissolved in the 160 c.c. of water at 86° F. The yeast is mixed in a mortar with 60 c.c. of the salt and sugar solution and added to the flour, the remainder of the solution being used to rinse out the mortar and then added to the flour with which it is mixed to form a dough; this is gently kneaded until it becomes uniform in texture and, exactly five minutes after the yeast was added to the flour, the rounded dough is dropped into the dough mould. The bar is adjusted across the mould, the time is noted and the test is placed in the incubator at 86° F.

Between fifty and sixty minutes later the rising dough should touch the bar, a point that may be determined by inspection or by electrical contact. Yeast that expands the dough in less than fifty minutes is fast, and one that exceeds sixty minutes is considered to be lacking in power.

Sugar Test. The fermenting power of yeast is defined as the degree to which yeast is able to decompose a certain amount of sugar in a given time. Attenuating power is measured by the amount of sugar which a yeast can decompose irrespective of time.

These properties of yeast are so closely related that the sugar-fermenting test is sometimes extended, as regards time, in efforts to determine the attenuating power of the yeast, but owing to the fact that equal volumes of water may be displaced in parallel tests of yeasts that differ widely in quality, the information gained is of doubtful value except as an indication of the protein content of the yeast.

In fact this feature is reflected in the result of the sugar test where it is found that the amount of water displaced by the CO_2 evolved during the fermentation is approximately proportional to the protein content of the yeast.

Protein content of yeast .	45%	50%	55%
C.c. of water displaced .	650	800	900

In the sugar-fermenting test the apparatus is so arranged that the volume of CO_2 evolved during the fermentation of a 10% sugar solution at 86° F. displaces an equal volume of water from a closed container; the quantity so displaced is noted at intervals of thirty minutes, and the total for two hours is accepted as a measure of the fermentative power of the yeast. The following apparatus is required (see Fig. 29):

A 750 c.c. glass jar with doubly perforated stopper furnished with a thermometer and tube connection to the aspirator flask.

A 2-litre aspirator flask containing water at room temperature covered with an oil film to reduce CO_2 absorption.

A water bath maintained at 86° F. by a heating element with thermostatic control, and a 750 c.c. graduated cylinder.

The sugar solution is prepared by dissolving 40 gm. of pure cane sugar in a small volume of hot distilled water and diluting this to 400 c.c. with distilled water at 86° F.

Ten grams of the yeast under examination is mixed with a little of the sugar solution in a mortar, washed into the jar

with the remainder of the solution and allowed to ferment for thirty minutes. The jar is then closed and the water displaced by the CO_2 is measured at thirty-minute intervals for two hours.

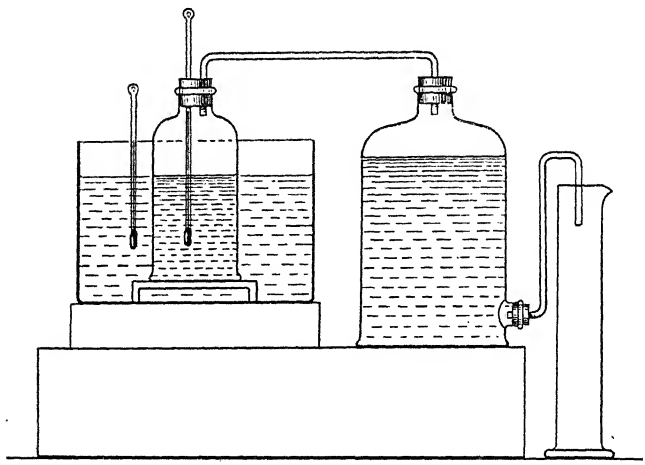

FIG. 29.

Practical Bakehouse Trial. When conducted under suitable conditions and skilled supervision this is the most convincing test of the baking quality of yeast; but the trial should be conducted in an automatic bakehouse where conditions must be of machine-like precision. In rare cases useful information is gained from tests conducted in a manually operated bakehouse; but they are more often quite unreliable, and a more satisfactory indication of yeast quality is gained from the routine laboratory tests.

Moisture in Yeast. The moisture content of commercial yeast varies between 72% and 73·5%, and is determined by air drying 3 gm. of the sample until it crumbles to a powder; evaporation is then continued in a hot-water oven for four hours, or until the weight is constant.

Ash in Yeast. The ash of yeast, and its constituents, is determined by the standard methods of food analysis and varies widely according to the raw materials used, and the type of brew in which it is grown. Yeast from molasses worts

may contain as much as 8·5% ash in the dry substance, but when it is grown in worts derived from cereals the ash is often less than 3·0%. The percentage of ash appears to have no influence upon the fermenting power or durability of the yeast.

Nitrogen in Yeast. The nitrogen content of yeast is determined by the Kjeldahl Gunning method.

Three grams of yeast are placed in a 500 c.c. Kjeldahl flask with 10 gm. of potassium sulphate, 20 c.c. of concentrate sulphuric acid, a small crystal of copper sulphate, and digested by placing the flask in an inclined position over a small Bunsen flame. When frothing ceases the heat is increased and digestion continued until the liquid becomes clear and nearly colour-

The flask is allowed to cool and the contents diluted by the cautious addition of 200 c.c. of water, sufficient soda lye to make the solution strongly alkaline is poured down the side of the flask so that it does not mix at once with the acid solution. The flask is attached to a condenser, shaken to mix the contents, and the ammonia distilled until about 150 c.c. of the distillate has condensed into 20 c.c. of N/2 H_2SO_4 contained in the receiving flask.

The acid is titrated with standard soda and methyl orange indicator. Each cubic centimetre of N/2 H_2SO_4 neutralized by the ammonia is equivalent to 0·007 gm. of nitrogen. The result is expressed in terms of the percentage of nitrogen, or protein (nitrogen × 6·25), in the dry substance.

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TABLES